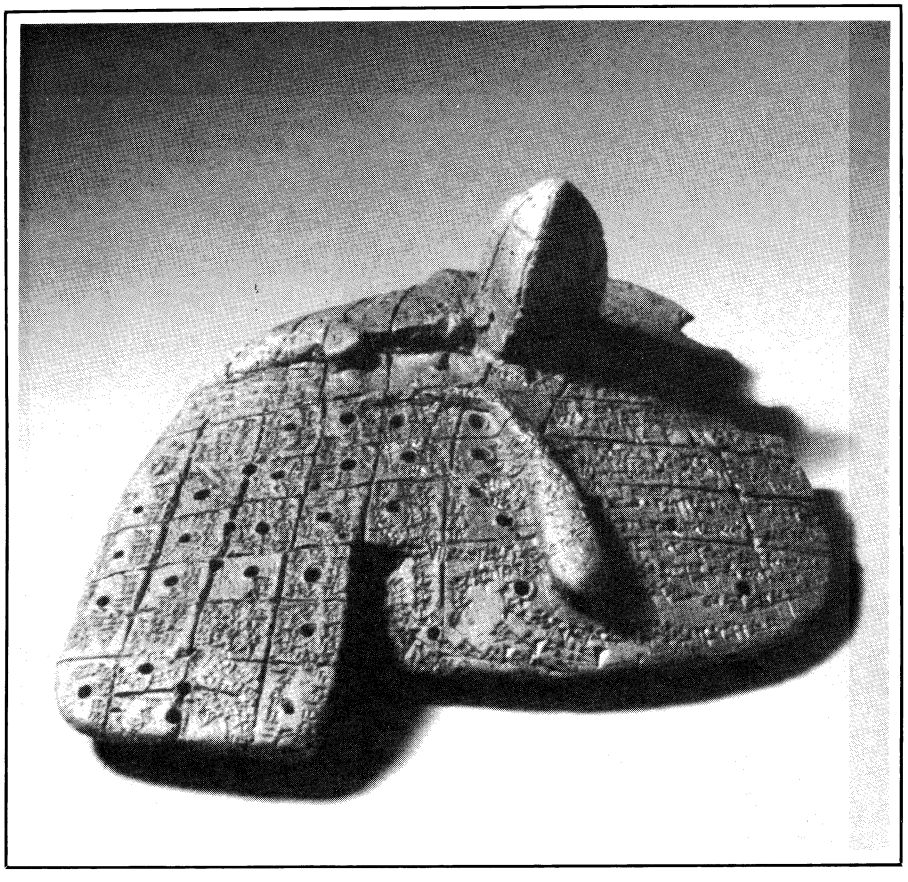
CLIO CHIRURGICA
LIVER TRANSPLANTATION



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Photograph (1968) of a dog whose orthotopic liver transplantation was carried out in the spring of 1964. The animal died of old age after 11-2/3 postoperative years.

Attending the dog is Mr. Paul Taylor. Taylor became the first organ transplant coordinator and procurement officer, creating a template from which hundreds of copies were later struck throughout the world.

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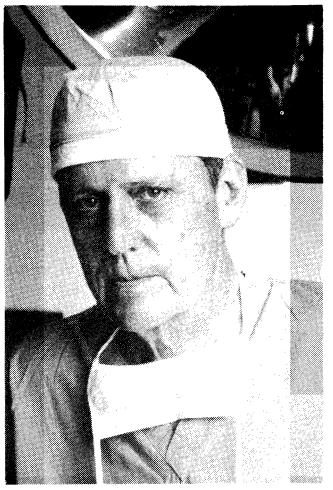
To Joy, Birgit, and Cynda



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Preface

Of the volumes of the *Clio Chirurgica* series, this one on liver transplantation will be the least encumbered by antique roots. The first mention of liver transplantation in the scientific literature was by Welch in 1955.[1] In his compendium of transplantation lore and literature, Woodruff[2] summarized what was known about hepatic transplantation to that time on less than two pages and with only two citations from the literature, including Welch's. Yet, less than nine years later, I authored a 545 page text on liver transplantation[3] by which time 299 articles had been published on this subject.

Almost all of the workers who have contributed to liver transplantation in its 32-year history still are living and most still are active. The hazards are obvious of assessing from this foreshortened perspective the contributory events to, much less the overall significance of, a new field. The advantages also are obvious. It was possible for me or one of the other editors personally to discuss with almost all of the authors the work represented in the key articles selected for this volume. Those who had died were well enough remembered to allow accurate reminiscences of their lives and times. Some of these memories are amongst the happiest that I have had, and some are amongst the saddest.

In the spring of 1987, I was approached by Dr. Ronald Landes concerning the preparation of this book. Originally, *Clio Chirurgica: The Liver* by Professor James Robinson (F.R.C.S., Eng.) was to have included a section on hepatic transplantation. However, both Dr. Landes and Professor Robinson realized that the material was too extensive and important to be an appendage to another subject. They proposed a separate volume, and Dr. Landes invited me to be the editor. The principal deterrent to accepting the invitation was my own role in the development of the field. I realized that almost all of the key steps in developing liver transplantation had been taken by three teams: Moore's in Boston; Calne's in Cambridge and London; and my own in Chicago (Northwestern), Denver and finally Pittsburgh. Subsequently, in mid-June, I met Roy Calne in Linz, Austria and discussed the matter with him. In September, I talked more briefly with Francis D. Moore. Encouraged by their comments, I decided to go forward. At this time a final preparatory step was taken with the recruitment of two co-editors who were chosen to bring to the task a more international perspective. The first, Carl-Gustav Groth of Stockholm had been a leader in the first successful trials of clinical liver transplantation in Denver. In the summer and autumn of 1967, he personally cared for these little recipients with a passion and tenderness that I never will forget. Dr. Groth now is Professor of Surgery at the Karolinska Institute and Chief of Transplantation at the Huddinge Hospital.

The second co-editor is Dr. Leonard Makowka, a 34 year old Canadian surgeon who now is Associate Professor of Surgery at the University of Pittsburgh and one of the most active workers in liver transplantation. Dr. Makowka is the complete modern surgeon with a remarkable array of clinical talents combined with a powerful background in basic science including a Ph.D. With the articles that have been selected, a reasonably coherent picture can be constructed about the development of liver transplantation. For each of the sections, an overview first will be given, often with more complete reference to the literature than represented by the articles selected for reproduction. In this way, the reader who wants to work back from the reproduced articles can move to the overview section first and from this second level can track back through the more complete literature as desired.

What may not be evident from perusal of the papers is the impact of transplantation on the field of hepatology. The story has unfolded since. Transplantation has made possible a fundamental philosophic departure in the way that health care is delivered. Until 50 or 60 years ago, practitioners of medicine, powerless to provide much more than sympathy, observed and presided over lethal disease of vital organ systems. Even with increasingly specific drugs, a rear guard strategy was all that could be offered for most organ specific chronic disorders. Patients with a failing liver could be treated with diet, medicines or a few palliative operations of questionable benefit. With the advent of hepatic transplantation, it became hypothetically possible for the first time in human history to provide exactly what was needed, a completely new liver. How high the stakes were or could be was well understood by those whose earlier written words are preserved in this volume.

But immunosuppression was too poor to apply this thrilling concept widely until the 1980's. Then, with the improvements and cumulative progress described in this collection of articles, it became

obvious that all future judgement in the care of liver diseases would have to be in the new perspective of possible eventual liver replacement. Mutilating operations in the portal hilum such as portacaval shunts or complex biliary drainage procedures for duct disease were virtually abandoned overnight in the 1980's since they jeopardized eventual candidacy for liver transplantation.

Hepatic transplantation also had an extraordinary effect on both basic and clinical research. Although all liver transplantations are therapeutic, every such operation also is an incisive experiment in basic physiology. With the treatment of some inborn errors of metabolism such as Type I Glycogen Storage disease, cure of the disorder by provision with the new liver of a known missing enzyme was highly predictable. In other disorders such as Wilson's disease, classical hemophilia, hypercholesterolemia and protein C deficiency, more was learned about the true nature of the original disease by the discriminating study of a single human liver recipient than by hundreds of prior investigations.

Finally, such a simple question as what are the necessary conditions for optimal revascularization of a transplanted liver has led to a major breakthrough in an understanding of liver physiology and of the influence that the so-called hepatotrophic hormones such as insulin have upon liver structure, function and capacity for regeneration. This subject is being treated separately in Part IV.

Thus, transplantation in general and liver transplantation in particular became a Pandora's box of 20th century science.

Thomas E. Starzl, M.D., Ph.D
Pittsburgh, Pennsylvania
October 22, 1987

1. Welch CS: A note on transplantation of the whole liver in dogs. *Transplant Bull, 2:* 54, 1955
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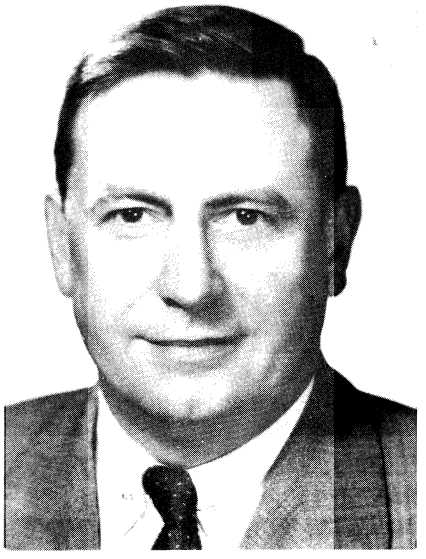

C. Stuart Welch (1909-1980). First man to perform experimental liver transplantation. Details about the man and the work are in Part IV.

Part I
Experimental Liver Transplantation, Exclusive of Immunosuppression

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Experimental Liver Transplantation, Exclusive of Immunosuppression

There are two general approaches to transplantation of the liver. With the first, orthotopic liver transplantation, the host liver is removed and replaced with a homograft. The alternative technique, auxiliary transplantation, is the insertion of an extra liver at an ectopic site. Because the orthotopic procedure has been by far the most important clinically, most of this volume will be concerned with this operation. However, auxiliary hepatic transplantation was the first liver engraftment to be attempted in animals and the amount of physiologic information that came from investigation of this operation was enormous. As will be described in Part IV, a new field was opened.

The distinction between creative and delusional thinking, if it can be made at all, usually becomes clear in distant retrospect. The idea of liver replacement first surfaced in 1956 with Cannon's publication[1] and was tested in dogs with increasing conviction and determination during the next five years. The canine model proved to be a difficult one technically, and systematic investigation of liver replacement was hampered seriously by the fact that it could be done successfully in only a few laboratories in the world.

The technical requirements for liver transplantation in dogs were almost too complex for simple categorization.[2,3] However, two cardinal requirements for immediate survival emerged. The first was adequate preservation of the homograft during its period of devascularization.[3] The second was decompression with veno-venous bypasses of the obstructed recipient splanchnic and systemic venous beds during the anhepatic period when the host liver was being removed and the new liver was being inserted.[2,3]

Rejection in the unmodified canine recipient. The clinical, biochemical and histopathologic events of liver rejection were described in the canine experiments performed during 1958-60 in Boston[2] and Chicago.[3-5] The earliest pathologic analyses were provided by Gustave Dammin of Harvard and Donald Brock of Northwestern University. In late 1961, the Northwestern University program was moved to the University of Colorado where pathology support was given by Rollo Hill and David Rowlands. Beginning in the autumn of 1963, specimens from Denver were sent to Professor K.A. Porter of St. Mary's Hospital and Medical School, London. The collaboration between clinicians at the University of Colorado (later Pittsburgh) and Porter in London has lasted for a quarter of a century. The initial linkage was made in September, 1963 at a meeting at the National Science Foundation in Washington, D.C. on the subject of renal transplantation. Porter came to Denver after that meeting with the objective of examining grafted kidneys and left with a commitment as well to experimental hepatic pathology that was to last for the next twenty-five years.

Porter's subsequent descriptions of the pathology of liver homografts in treated and untreated animals and in humans were classic. With the exception of a book chapter[6] and a monograph[7] published in 1969, these descriptions were parts of multi-faceted publications under the first authorship of surgeons.[8-11] One such study correlated for the first time the alterations in hepatic blood flow with light microscopic and ultrastructural changes of rejection.[9]

In 1965, orthotopic liver transplantation in the pig was described in France.[12] Within a few months, the advantages of pig liver transplantation were exploited by two British teams,[13,14] and, subsequently, many noteworthy fundamental studies have been published with this model.

Graft preservation. While studying total body hypothermia for cardiac surgery in the 1950's, Swan and Owens observed that lower temperatures protected the kidneys and other abdominal organs from the injury of cross-clamping of the thoracic aorta.[15] This advantage of renal cooling was confirmed in simpler ischemia models.[16] When Lillehei and his associates began their attempts at bowel autotransplantation and homotransplantation in 1958, they cooled the intestine by immersing it in cold electrolyte solution.[17] Refrigeration through the thin-walled bowel almost was immediate.

"Core-cooling" in transplantation using chilled lactated Ringer's solution for intraportal infusion was introduced during the efforts at liver replacement in 1958 and 1959[3] It was natural to use the same methods to cool kidneys through the renal artery[18] and eventually core-cooling was adopted

as the initial step in the procurement and preservation of all organs.[19] Cold infusates other than lactated Ringer's solution have been used over the years for renal homografts. These have included low molecular weight dextran[18] and solutions containing high potassium and high magnesium concentrations[20] simulating that inside cells (Collin's-like solution).

In 1975 and 1976, Benichou et al[21] in Denver and Wall et al[22] in Cambridge showed that with Collin's solution and with a plasma-like solution, respectively, dog livers could be preserved for 12 hours or more. It became possible to ship livers from city to city instead of relying upon the previous practice of having the donor in the same hospital as the recipient. Recently, Jamieson et al[23] at the University of Wisconsin have been able to preserve canine livers reliably for 24 hours with an even more effective cold solution that holds promise of expanded organ-sharing between regions and countries, or even transcontinently.

The alternative to infusion and cold storage is continuous perfusion. In a prototype of many later efforts, Marchioro et al[24] attempted whole body or regional cadaveric perfusion in dogs using a pump oxygenator into which a heat exchanger was incorporated for cooling. Subsequently, *ex vivo* perfusion of dog livers for 24 hours was accomplished by Brettschneider et al[25] using whole blood and a simple oxgenerator that was housed along with the grafts within a hyperbaric oxygen chamber as described earlier for kidneys by Ackerman and Barnard.[26] Although several human livers were preserved with this method, the complexity of the approach and the potential dangers of the high compression oxygen chamber caused the technique to be abandoned.

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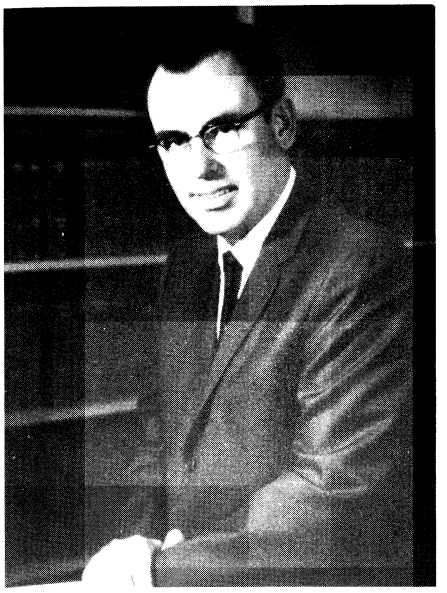


Jack A. Cannon

The first known efforts at experimental orthotopic transplantation of the liver were made by Dr. Jack Cannon who at that time was working at the new Department of Surgery, University of California, Los Angeles (UCLA). It was suspected that the liver might play a role in rejection. Cannon apparently hoped that a hepatic homograft would be more kindly received than other transplanted organs since it presumably would not contribute to its own rejection. The article did not have a title and descriptions of Cannon's procedure or even of the animal used. "Several successful operations" were mentioned but without survival of the recipients.

Even for those with no interest in history, Cannon's one-page article may have the special fascination of the nearly empty canvas upon which a complex mural was to quickly and unexpectedly appear. As summarized by Cannon only 32 years ago, in 1956 there was no identifiable reason to hope that any whole organ could be transplanted successfully, including the kidney, much less more complicated grafts such as the liver, heart or lung. Cannon's principal co-worker at this time was William P. Longmire, Founding Chairman of the UCLA Department of Surgery. In 1956, Cannon hired Paul I. Terasaki to be one of the "departmental" immunologists. Terasaki became the father of clinical tissue typing.

Brief Report

Transplantation Bulletin 3: 7, 1956

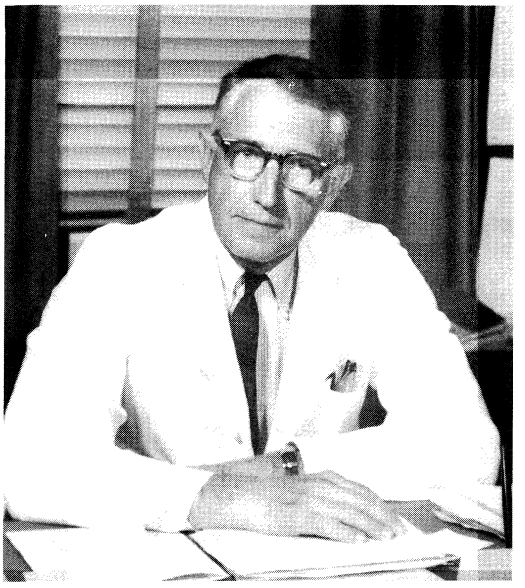
Jack A. Cannon

Although our activities in the Tissue Transplant Laboratory at the University of California Medical Center, Los Angeles, in the past months have been distinctly curtailed due to problems of activating a new physical plant, we nevertheless have conducted scout experiments along two avenues of research in the field of tissue transplantation.

It has seemed to us that there are two main areas where tissue transplantation may have wide clinical application. The first is in the transplantation of specific living tissues: skin, endocrines, bone, etc. The second is in the replacement of specific organs such as liver, lung, kidney, heart, or bowel. Admirable progress has been and is being made in the field of tissue preservation and storage. Little has been done in the investigation of the possibility of organ preservation or storage.

It is our belief that a relatively simple organ, such as the lung, might lend itself to eventual preservation for significant time periods if the organ could be provided with artificial circulatory, respiratory, and homeostatic functions. In addition, in such a set-up, significant measures might feasibly be directed toward despecification of the organ, or toward obtaining compatibility with a given future host. With this concept in mind we have attempted to maintain circulation and respiration in excised whole dog lungs using a small pump to perfuse donor blood through the lung and a small automatic respirator valve to provide respiratory function. Even with crude apparatus, we have found it possible to maintain circulation and respiration with satisfactory oxygenation in isolated dog lungs for several hours. We believe that with properly constructed apparatus to provide control of pressure, humidity, temperature, respiratory exchange and circulation, such an organ might be maintained for many days. Many interesting avenues of investigation might thereby be opened.

We have conducted further scout experiments in the field of organ transplantation. Removal and immediate replacement of a lung or kidney has been successfully accomplished by many investigators. Considerable work has been reported on the response of homografted lung, kidney, and heart. It is apparent that such transplantation fail, not because of technical fault but because of the homograft reaction. Consequently mere transplantation of organs without some attempt at modification of the reaction cannot be expected to lead to encouraging results. On the other hand, the liver undoubtedly has a great deal to do with the production of the homograft reaction and probably with the inception and maintenance of tissue specificity. Replacement transplantation of intact liver, therefore, might well lead to interesting results. We have read with very great interest the report of Dr. C. S. Welch in Volume 2 (2) of the Transplantation Bulletin. We infer from his communication that whole liver was transplanted to a host whose own liver was left intact. The reported result is that the expected homograft reaction occurred. We have been interested in the possibility of replacement of the entire liver with an intact liver homograft. We have so far performed several "successful operations" without survival of the "patient." At present we are attempting to determine whether, in our hands, excision and autograft replacement of the liver is feasible. We have been using mild hypothermia, but as yet have had no survivals. We will be greatly interested in, and appreciative of, any comment or discussion pertinent to this particular problem.



Francis D. Moore

In June of 1958, Dr. Francis D. Moore at the Peter Bent Brigham Hospital, Boston began a well-organized program of orthotopic transplantation of the canine liver. Dr. Moore was Moseley Professor of Surgery at Harvard. He had been one of the driving forces of the epoch-making renal transplant program at that institution. Moore's career and productivity were on such a grand scale that one almost could imagine his accomplishments to be fictional were they not so easily verifiable. Of all of Moore's contributions to surgery and science, his descriptions of canine liver replacement will be judged by many to have been his most important. Moore presented his work at the American Surgical Association where it was discussed by Starzl of Northwestern University in Chicago.

Experimental whole-organ transplantation of the liver and of the spleen

Annals of Surgery, 152: 374, 1960

F. D. Moore, H. B. Wheeler, H. V. Demissianos, L. L. Smith, O. Balankura
K. Abel, J. B. Greenberg and G. J. Dammin

Introduction

The rapidly growing experience in experimental homotransplantation is largely based on observations of the skin, kidney, blood and bone marrow.

These studies have been the pioneer ones for reasons that are clear historically: skin for its availability; kidney for its significance as a paired organ with a simple vascular pedicle, the biochemical product of which can easily be measured; blood for its circulatory support; and bone marrow for its use as a replacement tissue after hematopoietic destruction either in cancer treatment or as a preliminary to other transplants.

Experiences with these tissues may give us but a partial view of the total histologic phenomenon. Neither skin nor kidney is itself importantly involved with the production of immune globulins or mononuclear cells of the reticulocyte-lymphocyte-plasma cell series. While the bone marrow is involved with both, it is difficult to recover for microscopic study after injection and its study is confined to episodic examinations of peripheral areas where it alights and grows. Even in these areas, its identity is usually established by its peripheral product, rather than its local appearance. Studies of other organs and tissues are therefore of great interest in this field to obtain a broader and more complete picture of the biological response to homotransplantation. Organs which would particularly appeal as a way of broadening our view of the homotransplant phenomena would be organs involved either with a large antigenic mass, or those containing immunologically competent cells. Examples of such are to be found in the liver and in the spleen.

In the present state of our knowledge, clinical homotransplantation must rest on a state of immune tolerance achieved by chimerism, in which donor cells reside in the host without exciting an effective immune response. If such can be attained by destruction of the immune system (by irradiation or cytotoxic drugs) followed by hematopoietic restoration (by bone marrow or, in terms of the work herein reported, spleen), then the recipient can accept and hold other tissues from the same donor whose cells are responsible for the hematopoietic restoration.

The Liver

The liver represents the largest single homogeneous antigenic mass of cells that can be readily transplanted in the mammal. Its parenchymatous cells are homogeneous structurally and are importantly involved in metabolic work. Their metabolic efflux can readily be studied and identified in both bile and blood. The liver is concerned with the immune process to the extent that the reticuloendothelial system finds representation in the Kupffer cells lining the hepatic sinusoids.

Technics of transplanting liver tissue to an animal maintaining its own liver have been developed.[2,9] Studies of the transplantation of small liver slices might hold some promise. In these settings, the acceptance of the transplant is not itself essential for survival of the organism, and one cannot study the effect of the intact transplanted whole organ on the bodily economy. The liver, for its very size and complexity of vascular arrangement, does not lend itself to transplantation to some other portion of the body. It will clearly rest most comfortably and function most normally if placed in its normal site with anastomotic restoration of its anatomic relationships, unharmed by vascular deprivation, and free of distortion of its enteric relationships through any kind of vascular shunt. For these reasons, our initial purpose was to transplant the intact liver into its anatomic subdiaphragmatic site.[6,7]

The short-term hepatectomy suffered by the recipient dog was not anticipated as a problem and did not turn out to be so. The transient anoxia of the transplanted liver could indeed be a major problem had it not been for the striking demonstration[5] that liver, of the various tissues of the body, most outstandingly exhibits the phenomenon of cellular integrity while anoxic, if subjected simultaneously to hypothermia.

Looming larger as a barrier than any of these problems in hepatic transplantation was the circulatory instability of the dog who has no liver in place. Since the liver is removed together with the vena cava this dog suffers from pooling of blood in the kidneys, gut and lower extremities. If simultaneous aortocaval occlusion is instituted for a prolonged period, a severe acidosis of the distal portion of the dog is produced which will result,

upon reopening of the occlusive clamps, in a severe hyperkalemic hypoxic acidosis, cardiac arrhythmia, shock and death. This problem was solved by the use of two low-pressure shunt systems from lower caval and portal systems, respectively, to the jugular veins.

The dog was selected for this work because of his size, the lack of any evident inbreeding, and the extensive experience previously gained in these laboratories with kidney transplantation in the dog. Unfortunately, the dog's tissues harbor anaerobic organisms and for this reason control of infection, both in the liver, in the gastrointestinal tract, and other tissues, is essential for such an operation that may be associated with hypoxia or shock. Antibiotics were used throughout.

1. Operative Procedure in Hepatic Homotransplantation.

a. Experimental Group. The steps involved in the development of this operation will not be reviewed in this paper. The procedure currently used, and that employed in its essentials for all of the animals later described, will be briefly recounted.

Two dogs of approximately equal size and of either sex were used. Early in our experience the dogs were treated preoperatively with antibiotics given by mouth but subsequently these were not commenced until the day of operation.

Anesthesia is induced with intravenous barbiturate and the dog ventilated via a cuffed endotracheal tube. A minimum amount of anesthetic agent (ether) is used. Continuous monitoring of arterial pressure is maintained throughout the operation. High positive pressures are avoided in the airway.

The recipient animal is operated upon while normothermic. It is possible to do the entire operation without opening the thorax, though many of our earlier experiments involved a short intercostal incision on the right for the upper caval anastomosis.

The vena cava below the renal veins is isolated for the insertion of one of the two temporary shunts. The right adrenal is then dissected off the vena cava above the renal veins, the structures of the porta hepatis are identified, dissected free and all save the hepatic artery and portal vein are divided. Prior to this dissection, 2 per cent procaine is infiltrated around the hepatic artery and in the structures of the porta hepatis, a feature that appears to prevent hepatic outflow obstruction, to which the dog is so prone. It appears to make little difference to the animal's course whether or not the spleen is removed. In the earlier series it was removed, while in all of our recent animals the spleen has been left in place.

Attention is then turned to the suprahepatic area. The vena cava is dissected free of the diaphragm. It is possible to dissect it completely free at the diaphragm and, with care, it is possible to avoid entering the pleura. Isolation and division of the left phrenic vein is the most troublesome detail of this dissection.

The aorta is dissected free above the celiac axis for temporary occlusion during transient portal vein occlusion before its shunt is opened and later during anastomosis. A stainless steel T-tube is then placed in the lower vena cava, and the shunt (1/4 inch internal diameter) from the cava to the right jugular vein is placed. The liver is then ready to be removed.

The portal vein is then divided and the portal blood shunted to the left jugular vein by a flexible plastic ("Tygon") tubing (5/32 inch internal diameter).

During this procedure, a simultaneous operation has been under way on the donor dog. The steps taken are the same as those recounted above save for the fact that no preparation for a caval shunt is needed. When the liver is freed up, the animal is made hypothermic by the application of cold isotonic salt solution to the peritoneal cavity. We have also used immersion and portal perfusion of the liver for cooling after it is removed from the animal. The donor operation should be conducted so as to provide a liver whose edges are sharp, smooth and pink and that does not show any of the dark rounded swelling characteristic of canine hepatic outflow obstruction. When the liver is cooled to the neighborhood of 28° C. or lower, it is taken out with adequate segments of its vessels, and placed in the recipient dog.

The resuture of this liver in the recipient is then carried out anatomically while the two shunts maintain venous return to the right heart. First the inferior vena cava above the liver is sutured end-to-end. Then the portal vein is sutured end-to-end. Upon opening the portal vein anastomosis the clamp above the liver is released so that perfusion of the liver now begins with the recipient's own blood via the portal vein. The liver soon becomes quite nomal in appearance if the donor operation has been gently done. The total "dead time" of this liver is approximately 30-45 minutes up to this point. The cava below the liver is then anastomosed. The hepatic artery is then sutured end-to-end after gentle dilatation of the two ends. As soon as the vena cava is opened, the caval shunt can be removed. The bile ducts can be dealt with in one of several ways. The most satisfactory consists in cholecystduodenostomy.

The incisions in the neck and the abdomen are then closed. No steps are needed to maintain the liver immobile in its position. It seems to be held very firmly by its anastomotic arrangements and by the pressure of the surrounding viscera.

Transfusions are given as needed, with the use of ion-exchange blood during the anhepatic phase. Acidosis, developing during transplantation, is effectively treated by sodium bicarbonate given intravenously.

No heparin is required for the maintenance of shunt-flow in the anhepatic dog. If the transfusion volume is large the dog will be found to have a coagulation defect at this time that makes hemostasis difficult. When the operation is carried out with more dispatch and with less bleeding, no coagulation defect is identifiable by clotting time, prothrombin time, recalcification time and clot lysis tests.

The plane of anesthesia must be very light. It is desirable that the animal regain consciousness shortly after the operation. Animals that fail to do so often fail to survive the first 24 hours.

b. Control Group. Our control procedures have consisted of two types.

1. *Autotransplants.* In this procedure, the same operation as that described above for the recipient dog is carried out. The liver is lifted out of the animal, cooled in a bath of isotonic solution and by perfusion, using chilled oxygenated blood and is then replaced in the same animal.

2. *Liver dissections, or sham operations.* In this procedure, the liver

TABLE 1. *Liver Transplantation*
Gross Pathological Findings in Dogs Surviving Two Days or Longer

Animal	Day of Death	Pathological Findings
X-18	5	Atelectasis; small bowel intussusception; slightly swollen liver.
X-20	6	Multiple hepatic infarcts; hepatic artery thrombosis; bilateral hemothorax; atelectasis.
X-24	5	Multiple myocardial lesions; multiple gastric ulcers; pulmonary congestion and atelectasis.
X-28	4½	Hepatic infarcts; ? hepatic artery thrombosis; myocardial lesions; partial I.V.C. thrombosis.
X-35	6½	Hepatic congestion; gastric erosions; jaundice; atelectasis
X-36	4	Gastrostomy leak, peritonitis; myocardial lesions; partial hepatic artery thrombosis; focal pancreatic necrosis; renal infarcts.
X-44	2	Myocardial lesions; focal hepatic infarcts; focal pancreatic necrosis.
X-50	2	Hepatic artery thrombosis; hepatic necrosis.
X-58	12	Perforated duodenal ulcers, peritonitis; I.V.C. thrombosis (below renals); green, swollen liver.
X-69	8½	Small, pale liver; generalized jaundice; renal infarcts.

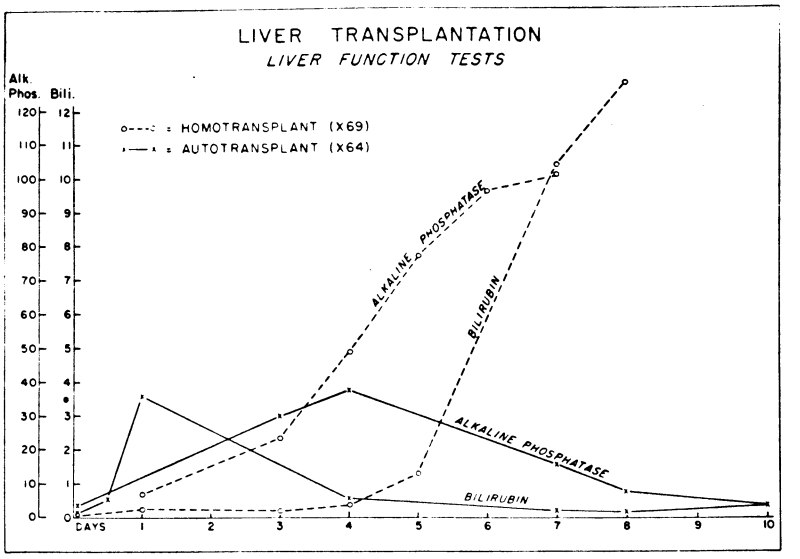


Fig. 1.— Liver function tests. Bilirubin and alkaline phosphatase in the autotransplant are only transiently elevated. In the homotransplant these values rise rapidly prior to death, the rise in alkaline phosphatase preceding that of bilirubin.

is dissected up, the shunts are placed, and everything is done in the way of dissection, including some of the vascular anastomoses, but the liver is not actually removed from the animal.

2. Results

a. Homotransplants. The homotransplant procedure has been undertaken in 31 animals. There have been 15 survivors over 24 hours. Eight have lived over four days, two have lived five days, and one each have survived 5-1/2, 6, 8 and 12 days. Our observations on clinical course, liver function and histologic change are based on the above animals.

1. *Postoperative Course.* After operation the animals are maintained on antibiotics; cortisone was used initially but was later not found to be necessary.

In animals surviving the first 24 hours, a characteristic clinical course was pursued. The animal regained consciousness, regained strength and ability to walk about. They recognized their handlers, had adequate urine flow, but in most instances were unable to eat significant amounts of food. A few dogs had biliary diversion through a cholecystostomy, and in these animals the bile output ranged up to 180 ml. per day. A number of animals succumbed during the first four days to anatomical complications of the

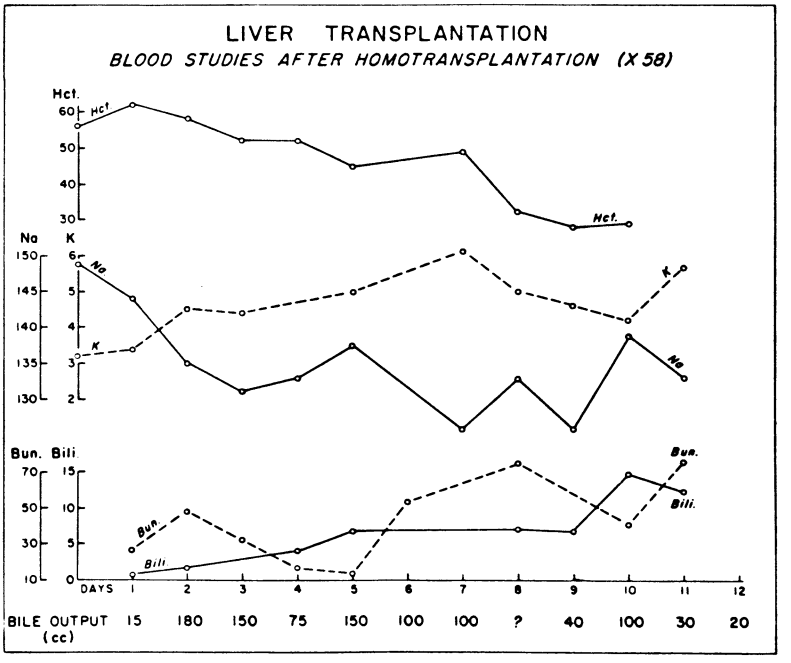


Fig. 2.— Hematocrit and blood chemical values. The first five days show a typical biochemical profile of a successful homotransplant. There is an early rise in hematocrit with later a gradual fall, postoperative sodium-potassium shift, transient rise in BUN with return to normal and an initially normal serum bilirubin which slowly rises. On the fifth day this dog developed an inferior vena caval thrombosis with partial occlusion of the renal veins. The subsequent chemical changes (particularly sodium, potassium and blood urea nitrogen) reflect the effects of this complication. There was perforation of a duodenal ulcer terminally.

procedure, including hemorrhage and embolus from the neck incision, and massive hemorrhage from gastroduodenal ulcer. In those that lived beyond this period, clinical and chemical hepatic function continued to within 24 hours of death.

Death was often unassociated with liver failure, was usually unaccompanied by hypotension or shock save terminally, and was presaged in a number of instances only by increasing jaundice, starting about 24 hours before death (see below). The fatal terminus did not resemble a fatality from hepatectomy in most of the animals, and was due to a combination of clinical conditions shown in Table 1.

2. *Chemical course.* In many animals the hematocrit was found to be significantly elevated during the first 48 to 72 hours. Three factors played a role here. One was loss of lymph into the peritoneal cavity, although all visible lymphatics were ligated in the porta hepatis. This was treated by infusions of saline or blood. In other instances, the elevated hematocrit was due to inadequately replaced water loss or to overtransfusion of the animal during the operation with subsequent plasma dispersal. If the transplanted liver developed outflow obstruction and became swollen and dark, a very considerable amount of blood was required to maintain the animal in a normotensive state; one to two days later this animal would be found to have a high hematocrit, suggesting that, as hepatic outflow tract obstruction was gradually released and splanchnic pooling abated, the animal became hypervolemic, lowering the blood volume towards normal by plasma dispersal and thus showing an elevated hematocrit. An almost unlimited number of chemical and hepato-functional determinations might be carried out in animals with transplanted livers. The following were selected as being of special interest.

The *blood urea nitrogen* was not significantly elevated in any of these animals save for one in which transient renal failure played a role. Nor was the blood urea nitrogen abnormally low. It remained between 10 and 25 mg.% in most of the survivors.

The *blood sugar* was usually normal or elevated. The hyperglycemia was not regarded as a significant finding because of the concomitant infusion of glucose in many instances. Occasionally hypoglycemia was noted during the first 48 hours if the intravenous infusion was stopped.

Alkaline phosphatase shows a progressive increase often antedating the rise in bilirubin concentration (Fig. 1).

The *serum bilirubin* concentration was generally normal or slightly elevated during the first few postoperative days but rose steadily prior to death. Five out of eight dogs who had this determination carried out 48 hours postoperatively showed values under 2.0 mg.%. By the fourth postoperative day only one of these eight dogs still had a bilirubin of less than 2.0 mg.%, and after the fifth day no animal had a serum bilirubin in this range. (Fig. 2).

The *Plasma albumin and globulin* concentration relationships were measured by paper electrophoresis. A fall in albumin and a rise in alpha-2 globulin was characteristic.

Coagulation studies (clotting time, recalcification time, prothrombin time and clot lysis) showed abnormalities in those animals early in the series in whom blood-trapping as unrelieved in limbs or portal circuits, or in whom hemorrhage had demanded large transfusion. As experience grew, no significant abnormalities in these parameters was observed until at or near the time of death when a bleeding tendency associated with a low prothrombin time became clinically evident.

3. *Histology* (Fig. 3-6). Anatomic findings at death, some of which were responsible for the fatality, are shown in Table 1. Isolated thrombosis in one or another of these vessels was not uncommon but massive thrombosis, either of the portal, caval or hepatic systems, was rare. In four animals pulmonary atelectasis was severe. In three animals major gastrointestinal complications would have been fatal without other disease.

The only organs showing pathognomonic pathologic changes were the liver and the heart. Where bile was diverted from the gastro-intestinal tract, gastroduodenal ulcer was common. The use of an entirely abdominal approach has reduced the incidence of pulmonary complications.

The histologic findings in the liver may be described as an initial infiltration of the portal areas and subhepatic veins with mononuclear cells, lymphocytes and plasma cells, with dilatation of the lymphatics. There is maintenance of the liver architecture with minimum distortion of the hepatic parenchymatous cellular mass and the sinusoids themselves. As the lesion progresses, the portal and centrolobular areas show progressively more cellular infiltration, finally including damage to the epithelium of the bile ducts. In one of the eight-day survivors (X-69) change in the cellular detail was seen in the parenchymatous areas. This was not seen in other dogs. This was unaccompanied by polymorphonuclear leucocyte infiltration or bile stasis and included vacuolization of cells and abnormal mitoses. If due to rejection, this response involved the parenchyma more than in any other dog. Interestingly, our 12-day survivor (X-58) showed less cellular damage than this eight-day survivor; only minimal necrosis was noted in the liver cells near the portal areas.

Early in our experience, lesions were encountered in the hearts of these animals. They were characterized by myocardial necrosis without cellular infiltration or visible thrombosis, going on to small areas of calcification. It was later shown that these can be produced in the dog by long, difficult operations, particularly those producing hypotension and requiring blood transfusion or by hemorrhagic shock. These lesions also occurred in the autotransplants.

b. Controls. Twenty-seven procedures for autotransplantation were instituted. Of these, ten survived 24 hours or more and seven, four days or more: two for four days, three for five days, one for ten days and one for 14 days. Causes of death were analogous to those in the homotransplant group, though the histologic appearance of the liver was quite different.

Of the "sham" dissections, 13 were done. Of these, nine were significant survivors and six lived14 days or longer, two being sacrificed at six and nine weeks, respectively.

The autotransplants were initially more difficult to accomplish than the homotransplants because there is less vessel-length for anastomosis

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Fig. 3.— Liver autotransplantation (X-35). Fifth day. There is dilatation of lymphatics and some interstitial edema. The parenchyma appears normal, and there is no mononuclear cell infiltration (Hematoxylin-Eosin, 100 X).

Fig. 4.— Liver homotransplantation (X-18). Sixth day. Marked mononuclear cell infiltration is present in the portal areas. There is some lymphatic dilatation. The parenchyma appears virtually normal (Hematoxyln-Eosin, 100 X).

Fig. 5.— Liver homotransplantation (X-69). Eighth day. The liver shows many morphologic changes in the hepatic cells themselves: necrosis, vacuolization, abnormal mitoses. There is also atrophy of bile duct epithelium and dilatation of the ducts. This animal showed a minimum of extraneous anatomical complications and a clinical picture most closely suggestive of purely hepatic disease (Hematoxylin-Eosin 430 X).

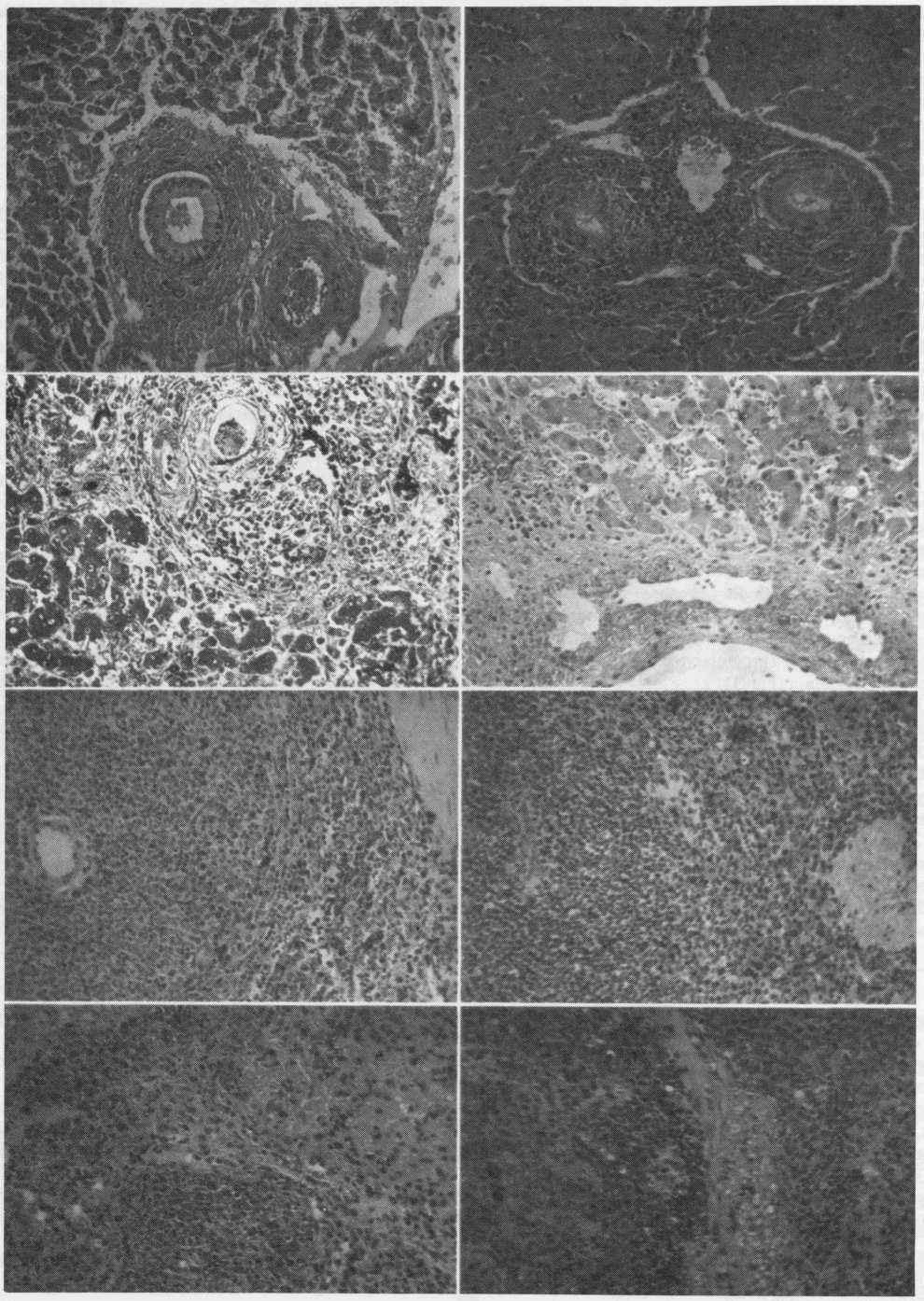
Fig. 6.— Liver homotransplantation (X-58). Twelfth day. There is mononuclear infiltration and lymphatic dilatation in the portal areas, but the parenchymatous cells are well maintained. In this animal death was due to perforated duodenal ulcer (Hematoxylin-Eosin, 100 X).

Fig. 7.— Spleen homotransplantation (S-12-A). Fourth day. There is a large active lymphoid follicle with many reticulum cells. The appearance is consistent with an immunologic response by the spleen. A few plasma cells are dispersed throughout the follicle (Hematoxylin-Eosin, 100 X).

Fig. 8.— Spleen homotransplantation (S-12-A). Sixth day. The follicle is packed with plasma cells and lymphocytes. No reticulum cells can be seen. Evidence of follicular activity has disappeared (Hematoxylin-Eosin, 100 X).

Fig. 9.— Spleen homotransplantation (S-12-A). Ninth day. The lymphoid follicle has undergone involution. No reticulum necrosis are now apparent in the adjacent red pulp (Hematoxylin-Eosin, 100 X).

Fig. 10.— Spleen homotransplantation (S-12-A). Ninth day. The red pulp is virtually replaced by plasma cell infiltration. Focal necrosis can be seen. There is intercellular vacuolization of the splenic trabecula (Hematoxylin-Eosin, 100 X).



and it is impossible to achieve parallel hypothermic arrangements for the anoxic liver without cooling the whole animal, a step that is definitely undesirable in this procedure. In those autotransplants and sham dissections that had prolonged survival, the most striking difference in contrast to the transplanted dogs were to be seen in their better clinical course, going on to normal dietary intake, and the finding of a liver which did not show the lymphocytic and plasma cell infiltration, degeneration of bile duct epithelium or hepatocellular damage. In common with the homotransplants, the autotransplants showed marked dilatation of the lymphatics in the portal areas and in the wall of the sublobular veins.

The Spleen

The nature of the histologic and clinical picture involved in homotransplantation of the spleen is a matter of concern because the spleen is an organ containing a large mass of immunologically competent cells. If grafted tissues react immunologically against the host such should be seen maximally in a structure such as lymph node or spleen. Beyond this lies the possibility that after whole body irradiation, hematopoietic restoration might be achieved by homostransplantation of the spleen from an unirradiated dog. The mass of whole spleen is many hundred-fold larger than any dose of cellular suspension or marrow aspirate that might be injected for such a purpose. The concept of hematopoietic restoration by intact spleen in whole body irradiation finds its basis in the work of Jacobson,[5] in which shielding of the spleen from whole body irradiation resulted in hematopoietic survival and clinical resuscitation of laboratory rodents after massive whole body irradiation. In Jacobson's experiments, the shielded spleen needed to be left in the animal but a short time to achieve its hematologic effect. In adults of other species (dog, man), the spleen does not normally harbor mature, active, hematopoietic tissue. It appears to be convertible to a multipotential hematopoietic organ as suggested by changes observed in such disease as agnogenic myeloid metaplasia or certain myelophthisic anemias in which the bone marrow becomes aplastic or is destroyed and the spleen becomes an active site of hematopoiesis.

The present experiments were undertaken to perfect a method for splenic transplantation, and to view the histologic and clinical results in the untreated normal dog.

1. Operative Procedure in Splenic Transplantation

Both dogs were operated upon normothermic and no effort at producing hypothermia in the transplanted spleen was made in most of the experiments. Experiments demonstrated that cooling by immersion or perfusion results in a rapid drop of spleen temperature, should such be shown to be necessary.

Before the spleen is removed from the donor, its vascular pedicle is isolated in such a way as to isolate the splenic artery near its origin as [sic] a point at which anastomosis can be done. When this artery bifurcates near the spleen the anastomotic procedure is facilitated. If its bifurcation is close to its origin, the operation is more difficult. In most of these procedures, the spleens were traded between two dogs. They were sutured in place by end-to-end anastomosis of artery and vein after gentle dilatation of the arterial ends. The dog's spleen is a large organ, occupying a larger fraction of the body weight and abdominal cavity than is the case in man. When transplanted to its normal site, it occupies a normal position free of torsion or distortion.

Control Autotransplants. In control animals, the spleen was removed and sutured back in place in the same dog.

2. Results

Splenic homotransplants were carried out in 29 dogs. In many of these animals (several of them early in our experience) the spleen did not become properly vascularized, and splenic hemorrhage or infarction caused the death of the dog. There were ten animals in whom good splenic vascularization was established as shown by gross and microscopic appearance at biopsy and by fresh arterial bleeding after the second postoperative day. Many animals underwent subsequent splenectomy if the condition of the spleen threatened their survival. The longest survival of spleen was in one animal at 65 days (S-12-B), established by biopsy and showing good vascular maintenance.

a. Clinical Course. If splenic vascularization was satisfactory, these dogs had no characteristic clinical disturbance after their operation. They were not carried on antibiotics and did not require intravenous therapy after the first day. If the spleen became necrotic due to distortion of its blood supply, the animal became ill. In those animals in which venous obstruction occurred with engorgement of the spleen, the animal might succumb to splenic rupture or hemorrhage, particularly after biopsy. Biopsies were taken at various intervals after the operation in both homotransplants and autotransplants.

b. Hematology. No characteristic hematologic changes (hematocrit, white count, smear) were identified in the peripheral blood of these animals. Leucocytosis was usually present, especially when the rejection response in the spleen seemed to be at its height.

c. Histology (Fig. 7-10). There was a striking difference in the histologic appearance of the homotransplanted and autotransplanted spleen. In those spleens that failed to achieve surgical viability early diffuse necrosis occurred, a picture contrasting with that of rejection.

The rate of histologic change is variable. As an illustration, the histologic sequence (slower than most) in homostransplanted spleen of dog S-12-A included the following:

1. (Days 2-4) Reticulum cell hyperplasia in large prominent active lymphoid follicles, compatible with a brief "graft vs. host" response.
2. (Well established on day 6.) Plasma cell infiltration, first in the follicles, then the red pulp and subcapsular areas.
3. (Well-established by day 9.) Disappearance of reticulum cells and involution of follicles with intercellular vacuolization in trabeculae. Diffuse massive plasma cell infiltration, particularly in the red pulp, with focal areas of necrosis.
4. Thrombosis of small vessels and complete infarction.

In all the other animals showing rejection, necrosis was established at the end of one week.

The course of one long-surviving splenic homograft is difficult to interpret (S-12-B). On day 36 there were follicles with active centers, present also on days 51 and 65. The cellularity of the red pulp was reduced in comparison with earlier time intervals. There is the possibility that chimerism may have been established. There is also the possibility that the cells seen in the follicles and red pulp represent a repopulation of donor spleen with cells of the new host.

Discussion

One-stage whole organ transplantation both of the liver and of the spleen may be accomplished in the laboratory. The transplanted liver survives and functions well for a time. Although we have had several survivors over 48 hours, we have had but a few animals whose surgical and clinical course was so smooth that one could feel confident that the clinical course was that of liver rejection. In such animals, the rejection process seemed initially to be focused largely on the portal areas leaving the parenchymatous liver cell quite free. This was reminiscent of kidney in which early rejection is directed at the tubular mass, leaving the glomeruli free of attack.

Future work on liver transplantation in the animal may take a number of different directions. The most important is a further extension of these experiments to view the unmodified rejection response more clearly. A second form of study will depend upon treatment of the recipient with radiation, radiomimetic drugs, with or without bone marrow or splenic transplant, to achieve a state of tolerance in which prolonged hepatic survival might be achieved. A third form of experiment would consist of transplantation into an animal in liver failure.

Looking to the clinical problem, it should be noted that the liver of a recently deceased animal appears to maintain viability so long as either the entire body, the liver perfusate, or the liver itself is rapidly cooled and maintained in the cool state.[8] Technics for the postmortem preservation of tissues will rest on the temperature coefficients for metabolic activity in each organ and the mechanical details of speed of cooling. The treatment of cirrhosis or other liver disease by hepatic transplantation offers promise clinically only where there is a comparative lack of conjoint pathology in other organs. The treatment of metastatic carcinoma by liver transplantation would offer salvage only in those rare patients where the liver was the only site of metastasis.

The splenic experiments demonstrate the histologic nature of splenic rejection and the fact that the rejection process seems to involve but a transient phase of graft reaction against the host.

Looking to the future of splenic transplantation in the laboratory, it is essential that experiments be carried out to discover methods[3,4] by which the spleen may be converted into a more active hematopoietic organ. Experiments of this type will involve whole body irradiation or preliminary treatment with radiomimetic drugs (both with splenic protection against the damaging agent). If the spleen can be converted to a more active hematopoietic organ, one may reasonably expect that after whole body irradiation or the use of radiomimetic drugs in the recipient animal, one may achieve splenic acceptance as has been the case with the skin grafts and bone marrow. Whether or not the destructive therapy required as a preliminary must be so severe as to endanger the life of the recipient dog can only be discerned by further study. In an animal harboring and accepting a spleen from a donor animal, one might hope to achieve an ideal situtation for subsequent acceptance of other organs because such donor would then be available for transplantation studies of skin, kidney, liver, or other organs. In this regard, if splenic hematopoiesis can be achieved, one has a more fitting donor-recipient relationship than is the case with the bone marrow transplantation. It is difficult to secure enough marrow from a single donor (particularly in man)[11] to repopulate the destroyed marrow of a potential recipient. Multiple-donor experiments are therefore of great interest.[10]

For any of these future possibilities to become a reality, the first two steps required are those of the development of a method for surgical transplantation of these two viscera, and the description of the normal course of transplantation without other treatment. Such has been the objective of the work herein described.

Conclusions

Experimental whole-organ homotransplantation of the liver and of the spleen has been carried out experimentally in the dog, transplanting these organs to their normal anatomical position and blood supply.

Control experiments have consisted of autotransplantation of the organs, returning them to their normal sites.

In both cases a satisfactory surgical technique has been achieved which produces a viable organ. Hepatic hypothermia is an essential feature of the liver transplant. Rejection of these viscera is a gradual cellular process, sharply to be differentiated from avascular necrosis or surgical failure.

In the case of the liver, a significant sparing of the parenchymatous liver cell mass is noticed early in the rejection process, the latter being directed initially entirely towards the portal areas and centrolobular veins.

In the case of the spleen, the rejection process is diffuse, results in atrophy of follicles (after an initial follicular stimulation), loss of follicular activity, and infiltration of the entire spleen with plasma cells that appear to arise from the recipient since there are no nests of active plasma-cell-producing areas in these spleens.

The implications of this work with respect to therapeutic transplantation of the two organs, and future directions for research in this field, are briefly discussed.

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Discussion

Dr. Stephen E. Hedberg: Dr. Cole, members of the Association and Guests. I should first like to congratulate Dr. Moore on the presentation of this interesting and excellent paper.

At Walter Reed we also have been interested in the transplantation of canine spleens and our work with transplantation of normal spleens to normal dogs has tended to confirm what Dr. Moore has shown you here today.

One of our aims in spleen transplantation has been to explore the possibility of protecting the recipient animals against high doses of ionizing radiation by means of a spleen transplant.

Very early in the game we satisfied ourselves that the normal spleen will not protect an irradiated recipient. Our protocol was therefore modified, and Dr. Moore thought that you might be interested to see what we are doing now and in hearing about some of our early results.

The first slide is a diagram of our experiment. Our donor dogs are females of D-negative blood type, immunized against typhoid and diphtheria and prepared according to a method outlined by Drs. Dunphy and Stanley Jacob. Two ribs are removed, the animal is given 150 r total body irradiation, and as soon as possible thereafter the ribs are returned to the spleen of the *same* animal.

Six weeks later these ribs, implanted in the spleen, are producing active bone marrow.

At this time a recipient dog, a male, D-positive blood type, immunized against tetanus, is given a supralethal dose of total body irradiation. The following day his spleen is removed and replaced by the marrow-containing spleen from the female dog. The male dog is then observed for prolongation of survival with respect to irradiated controls; his red and white cells are studied to note the appearance of female cells of each kind; his antibodies are studied with respect to typhoid, diphtheria and tetanus; routine hematologic work is done and biopsies are taken of his spleen at intervals after transplantation.

The next few slides are photomicrographs of sections from a spleen at the time of transplantation, and of the same spleen five days and seven days after transplantation.

This slide shows what the bone marrow looks like at the time of transplantation. This is essentially normal bone marrow and, outside the field of view it is surrounded by spleen that also looks quite normal.

The next slide - five days after transplantation - shows that tremendous hyperplasia has taken place in this marrow, apparently in response to the needs of the irradiated host. The next slide shows that changes have also taken place in the splenic pulp. This is a cluster of megakaryocytes — found in large numbers in this transplanted spleen seven days after transplantation, normally found only occasionally in the splenic pulp of the dog.

The final slide shows that, in addition to megakaryocytes, the splenic pulp is producing red blood cells; in the left upper corner are early red cell forms; you can see hemoglobin in some of these normoblasts.

I should be most unwilling to speculate what these findings mean or what they may promise, but certainly they are of interest to us and we intend to pursue them.

Again, I should like to thank Dr. Moore for asking me to elaborate a little bit on the subject of his stimulating paper.

Dr. Thomas Starzl: Dr. Harry A. Kaupp and I have been working on the problem of liver transplantation for the past 18 months. We have had

experience with about 80 hepatic transplants, but we were not able to obtain survival for longer than three or four days until we were encouraged by Dr. Moore's results in Atlantic City last Fall. Since then, our survivals have been longer.

(Slide) We have removed the entire aorta from the donor animal in continuity with the hepatic artery and liver graft enabling us to place a large vessel anastomosis in the abdominal aorta of the recipient animal. We have provided internal biliary drainage by a cholecystenterostomy below which is an enteroenterostomy.

(Slide) The venous reconstitution has been done in three ways. Shown on the left is the reverse Eck fistula type of reconstruction in which all of the blood from the inferior vena cava and the splanchnic system is deviated through the liver. This procedure has a very high immediate mortality, perhaps as high as 80 or 85 per cent due to inability of the liver to transmit the augmented venous return.

The second method we have used for venous reconstruction is shown in the middle. This consists of essentially anatomic reconstruction with the addition of a small portacaval shunt, so that if splanchnic hypertension or liver engorgement develops, decompression can occur. This is the most versatile method since very small dog's livers can be placed into large recipient animals.

The maximum survival with each of these two methods has been nine days. A third technic (shown on the right) has employed entirely normal anatomic reconstruction. In this case, the animals have had to be carefully matched as to size. With this method, the maximum survival has been 20-1/2 days.

(Slide) Shown here are some postoperative chemistries, and one can see that the chemical events parallel those demonstrated by Dr. Moore rather closely. The bilirubin is shown here rising as Dr. Moore demonstrated, usually starting up on about the fifth day. In this particular animal, our longest survival, the bilirubin rose sharply. At the time we thought the animal was just about to reject his liver, he began to recover. The bilirubin fell and was still declining at the time of his last chemistries, 18 days after surgery and 2-1/2 days before death.

We believe that many of our animals have died as a direct result of hypoglycemia between the sixth and tenth days. This animal manifested a fall in blood sugar which was around 25 or 30 for four or five days, but no intravenous glucose was given. He was allowed to eat. He was given sugar water to drink, and the blood sugar rose back to normal levels by the 15th postoperative day.

(Slide) As can be seen, other chemistries including alkaline phosphatase and cholesterol also manifested a return toward normal during the thrid postoperative week.

(Slide) This is a rather poor slide which shows that there is some liver tissue remaining and that there is massive round cell infiltration.

I would like to thank the Association for the privilege of the floor, and particularly Dr. Moore for the opportunity to discuss this fine paper.

Dr. W. V. McDermott: It is always very stimulating to hear Dr. Moore. We try to keep our inter-university across-town lines of communication intact, but sometimes they become fairly tenuous for periods of time. In our studies we have had no direct experience with homotransplantation of the liver, but in the course of some of our studies in the isolated perfused canine liver, replants have been done in order to continue observations initiated during the period of perfusion.

(Slide) In this experimental plan both the isolated liver and the donor animal are maintained during the perfusion. During a prolonged perfusion there are problems concerned with maintenance and restoration of the hepatectomized animal, but in three of the five attempts replants were successful after perfusion periods ranging from 1 to 2-1/2 hours. In all three survivors liver function, ammonium clearance and all standard parameters were entirely normal, and the longest survivor is now over three months.

One dog was sacrificed for the purposes of the study in progress. Microscopic examination showed an entirely normal liver. These limited observations would support Dr. Moore's observations that replant and transplant of the liver are technically and physiologically feasible and that the abnormalities described, particularly in the excretory functions of the liver, are a part of the rejection phenomenon and not due to any metabolic disorders attendant upon the operative procedure.

I certainly congratulate Dr. Moore on this beautiful presentation. I will be interested in his further studies.

Dr. Francis D. Moore (Closing): President Cole, I would like to thank Dr. Hedberg, Dr. Starzl and Dr. McDermott for their fascinating discussions. This new field that is opening up is a large one. There is a lot of work to be done in it, it takes long hours, lots of animals, lots of thought and will need maximal support from the national agencies.

Every time somebody comes to visit our laboratory they say, "Well, what good is it to study all this? Where are you going to get livers?" We have conducted a study, which I won't show you any slides of, on hypothermic preservation of the postmortem liver. To make a long story short, if the liver is cooled fast enough, it maintains excellent metabolic functional capacity up to 12 hours after death. This problem of a time-temperature coefficient in the postmortem survival of individual tissues represents an important horizon in this field. It is one that the Russians have explored with great interest. They have secured good kidney replants or auto-transplants after 28 hours of cooling. The source of tissue, as I think Dr. Cole intimated in his Presidential Address, is here, but there is much to be done in understanding how to use it right.

Finally, I would like to show my last slide, if I could (Slide) just to show you that there's nothing new under the sun. In 1909, Dr. Carrel with his penetrating insight realized that in exploring these things it was best to start with the simple things, so he began with replantation of the canine spleen.

Harry A. Kaupp, Jr. was a senior resident in the Northwestern University surgical program in 1958 and 1959 when the first work there with liver replacement was carried out in dogs. There were two residents assigned to the Surgical Research Laboratory, one to work in cardiac surgery and the other to work with one of the editors (TES), a junior faculty member. They flipped a coin and Kaupp lost. He became the second author on the first papers from Chicago on liver transplantation. After completion of training, Kaupp began a private practice of vascular surgery in Allentown, Pennsylvania. He retired in 1986 and is presently living in New Hampshire.

In July of 1958, experiments with orthotopic liver transplantation in the dog were begun at Northwestern University in Chicago simultaneous with and without knowledge of Moore's work. The absence of early communication between the Boston and Chicago groups was reflected in a number of differences in their techniques. However, the two central objectives of effective preservation of the liver and decompression of the obstructed splanchnic and systemic venous circulations during the anhepatic phase were the same. The principle of core-cooling used to preserve the liver grafts in the Chicago laboratories subsequently was applied to the initial cooling and preservation of all whole-organ grafts. The material from the Northwestern University studies was divided into two articles, the first essentially technical and the second describing the clinical and histopathologic events of rejection.

Reconstructive problems in canine liver homotransplantation with special reference to the postoperative role of hepatic venous flow

Surgery, Gynecology & Obstetrics, 111: 733-43, 1960

T. E. Starzl, Harry A. Kaupp, Jr., Donald R. Brock, Robert E. Lazarus and Robert V. Johnson

Although considerable experimental data have been accumulated concerning many organs, there have been few reports of homotransplantation of the liver. Until 1959, the only work had been done by Welch and his associates (9, 34), who were able to obtain function from liver homografts transplanted into the pelvis. Recently, Moore and his associates presented the first accounts of successful homotransplantation of the canine liver to animals with total hepatectomy (21,22). Maximum survival after operation was 12 days.

The techniques to be described for homotransplantation of the liver have previously been briefly outlined (12,28). The influence of portal flow upon the homografted liver has been analyzed in detail not only because this factor proved to be an important determinant of success or failure but also because the resultant information may have application in a variety of other experimental situations, including those involving hemorrhagic shock. Maximum survival after liver homotransplantation has been 20 1/2 days.

General Methods

Seventy-nine transplants were performed, using healthy adult mongrel dogs of 10 to 25 kilograms. In most cases, the weights of the donor and recipient animals were closely matched. Both dogs were anesthetized with 25 to 30 milligrams of sodium pentobarbital per kilogram and, after tracheal intubation, placed on respirators. The arterial pressure of the recipient dog was monitored during and immediately after operation with an arterial catheter connected to an aneroid manometer. Venous pressures were read directly from a water manometer attached to an indwelling catheter. The operation was performed under sterile conditions by two, or occasionally three surgeons. In analysis of results, the first 27 hepatic transplants were excluded from consideration because a variety of methods of liver preparation were used with a resultant inconstant quality of grafts.

PRELIMINARY STEPS IN THE RECIPIENT DOG

The abdomen was opened with an upper midline incision. A segment of aorta, 1-1/2 to 2 centimeters in length, was mobilized just above the inferior mesenteric artery for later anastomosis. Next, the mesentery of the caudate lobe of the liver was incised, and the inferior vena cava encircled with a tape, above the entrance of the adrenal veins. The gastrohepatic ligament to the left of the portal triad was then doubly ligated and divided. Finally, common duct and gastroduodenal artery were double ligated and divided as close to the duodenum as possible. The portal vein was cleaned off toward the liver until its bifurcation was encountered, taking care to ligate all lymphatics on its surface. When these steps were completed, the only uninterrupted structures remaining in the gastrohepatic ligament were the hepatic artery and the portal vein.

A portacaval shunt was then constructed in as inferior a position as was convenient (Fig. 1b). The exact size and technique of this shunt varied with the different techniques of venous reconstruction during implantation, but in every case the presence of the anastomosis was necessary for decompression of the splanchnic system during implantation of the liver. When the anastomosis was completed, the abdominal wound was closed with towel clips, and attention directed to the donor dog.

PREPARATION OF THE DONOR LIVER

During this operation on the recipient, the donor dog was immersed in an ice bath, and the body temperature reduced to 25 to 30 degrees C. The abdomen of the donor dog was opened through a long midline incision. The abdominal aorta was mobilized proximally to the level of the superior mesenteric artery, ligating and dividing all branches (Fig. 2). The superior mesenteric artery was encircled with a ligature but left intact for the time being.

Next, the stomach and spleen were retracted sharply to the right, and the celiac axis was dissected free from its origin to the trifurcation, where

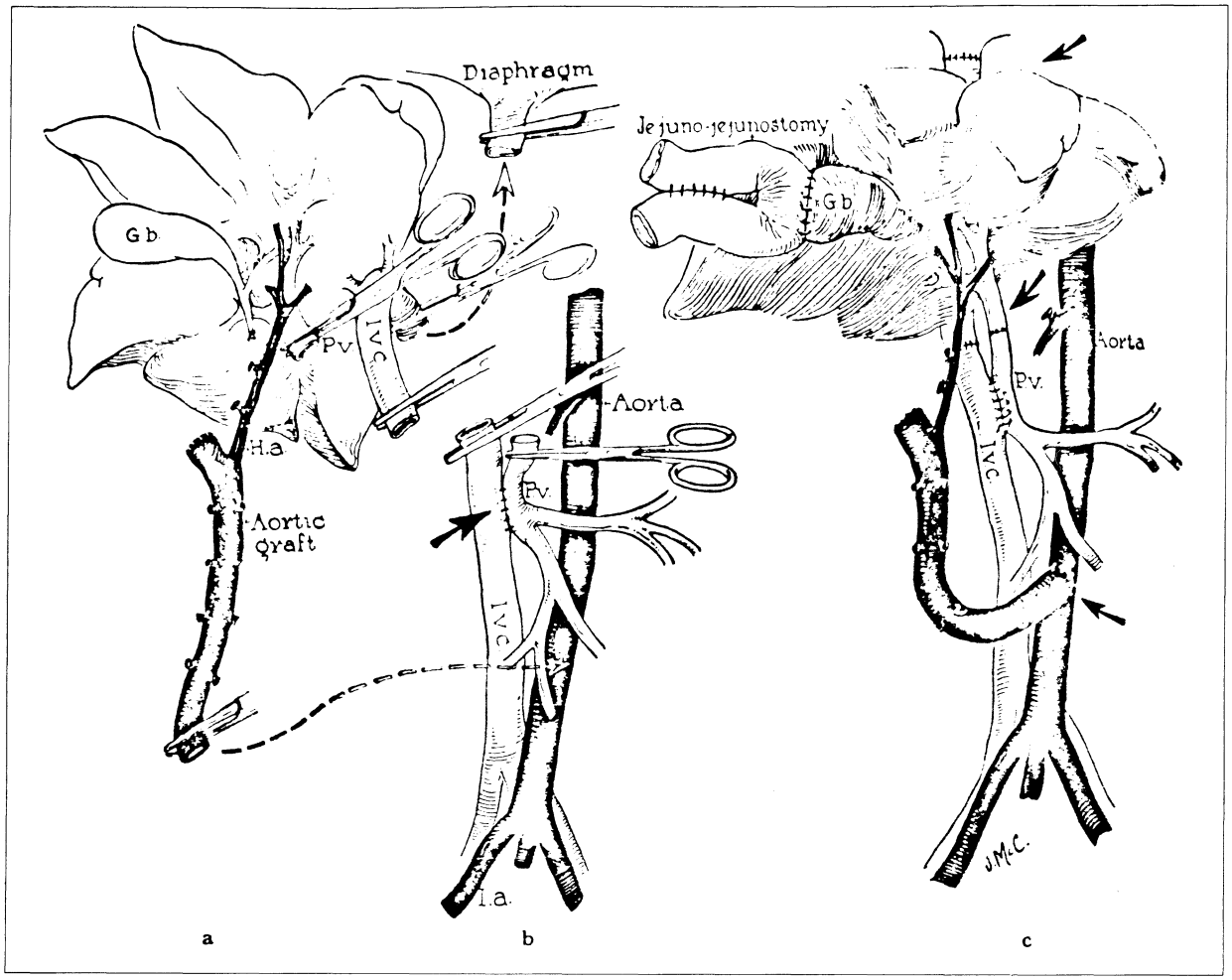

Fig. 1.— Basic technique of homotransplantation. a, Donor liver ready for transplant. Note aortic graft removed in continuity with hepatic artery and liver graft. b, Recipient with portacaval shunt and liver removed. c, Donor liver in place.

splenic and left gastric arteries were ligated and divided (Fig. 2). Attention was then directed to the portal triad. The gastrohepatic ligament to the left of the triad was first ligated and divided, and the common duct and gastroduodenal artery double ligated and divided near the duodenum. The portal vein was freed from its surrounding adventitia in which there were many lymph channels which required ligation. In order to obtain an adequate length of portal vein for subsequent anastomosis, the pancreatic vein was double ligated and divided. Several large lymph nodes usually still loosely connected the portal vein and hepatic artery inferiorly, but these were stripped out easily with blunt dissection, leaving the hepatic artery and portal vein skeletonized (Fig. 2). The donor liver was now ready for perfusion in situ.

The previously placed ligature around the superior mesenteric artery was tied. The portal vein was ligated as far inferiorly as possible, and the blunt end of a standard intravenous infusion set directed up the portal vein to the liver. Through this, 1,000 cubic centimeters of cooled (5 to 10 degrees C.) lactated Ringer's solution were used for gravity perfusion of the liver with a pressure head of 60 to 80 centimeters of water (Fig. 2). As soon as perfusion was begun, the animal was bled to death through a catheter inserted into the aorta (Fig. 2). The collected blood was subsequently used for transfusion of the recipient. During the perfusion, the interior of the liver cooled to 10 to 20 degrees C.

The liver was then removed after incision of the diaphragmatic ligaments by transection of the portal vein, the upper abdominal aorta, and the vena cava above and below the liver (Fig. 1a). The open upper end of the aortic graft was ligated. The vena caval cuff above the liver was carefully scrutinized for small holes which, if unrecognized, will lead to air embolus or hemorrhage after the liver is revascularized. A small incision in the gallbladder was made at the site of the proposed cholecystenterostomy to prevent autolysis. The liver was then brought to the table of the recipient animal.

Before evolving this method for preparation of the donor liver, a number of unsatisfactory techniques were employed. These failed generally either because of the use of heparin in the donor with consequent bleeding after transplantation or because the liver was not cooled enough to withstand the effects of ischemia.

HEPATECTOMY IN THE RECIPIENT DOG AND TRANSPLANTATION OF THE DONOR LIVER

After removal of the donor liver, the operating team returned immediately to the recipient dog and inserted an external polyethylene bypass from the femoral to jugular veins (Fig. 3), as described by Kaupp and Starzl (13). After ligation and division of the hepatic artery, the vena cava, above and below the liver, and the portal vein were grasped with Potts' clamps and the liver was removed (Fig. 3). A brisk flow through the external shunt caused decompression of the blocked splanchnic as well as of the caval beds.

The cooled donor liver was positioned in the upper part of the abdomen of the recipient animal (Fig. 1a and b). Anastomosis was performed with continuous No. 5-0 arterial silk between the cuff of recipient vena cava at the diaphragm, which was sharply pulled down to facilitate exposure (Fig. 4), and the short cuff of vena cava on the donor liver. Because of th limited space and short segments of vessels, it was necessary to sew the posterior row from within the vessels (Fig. 4).

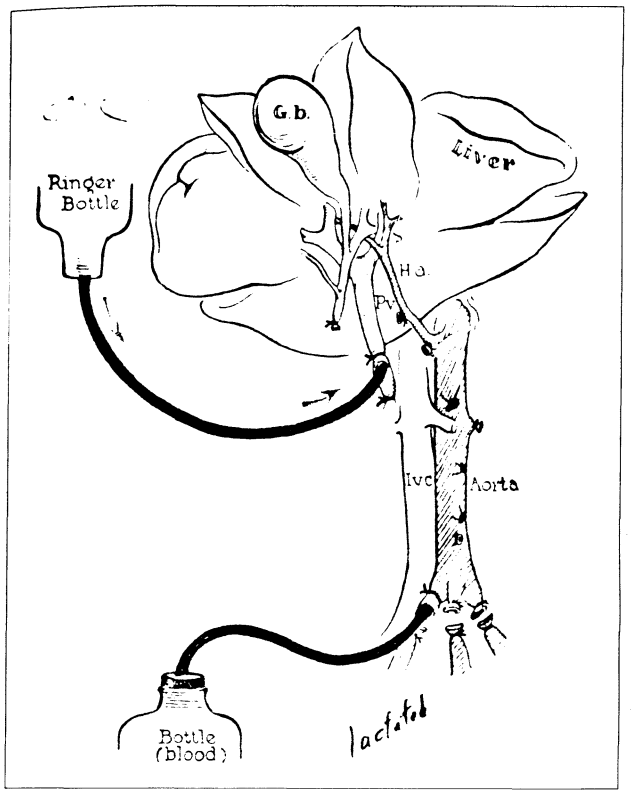

Fig. 2.— Preparation of donor liver. Aortic graft prepared. Perfusion of cooled Ringer's solution by gravity through the portal vein and collection of blood from the aorta.

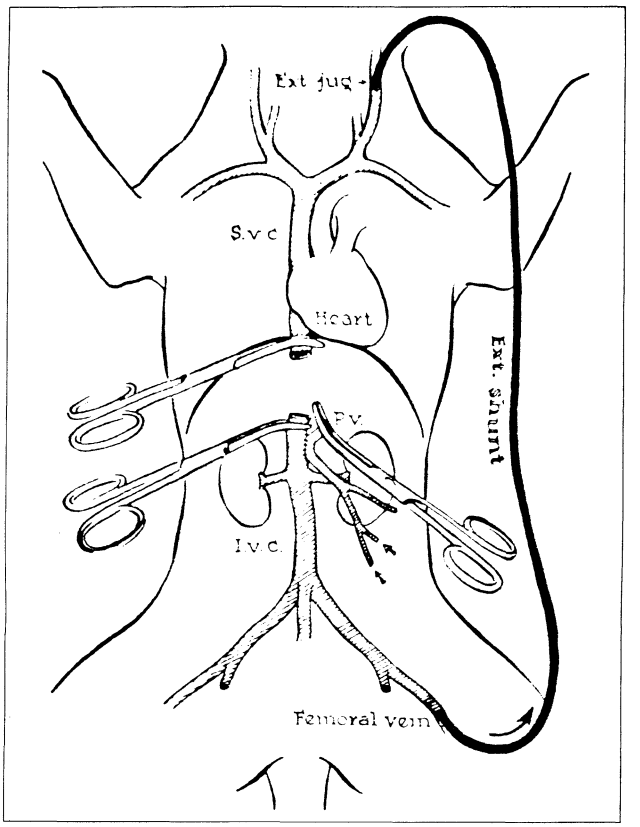

Fig. 3.—Decompression of portal and caval venous systems during complete occlusion of vena cava and portal vein by external shunt from femoral vein to external jugular vein.

Re-establishment of the portal and vena caval return was done in three different ways (Fig. 5). The choice of procedure influenced the type of portacaval shunt previously performed (during preliminary steps in the recipient dog). The first method of venous reconstruction employed the principle of the reverse Eck fistula (Fig. 5a), in which the preliminary portacaval shunt was made at least 1 centimeter in length. After completion of the caval-caval anastomosis above the liver, an end-to-end portal-portal anastomosis was performed, and the open stumps of donor and recipient vena cava below the liver were ligated (Fig. 5a). With release of the clamps, the venous return of both the caval and splanchnic systems were directed through the liver (Fig. 5a).

The second method resulted in anatomic venous reconstruction with the addition of a small portacaval shunt (Fig. 5b). In this case, the preliminary portacaval anastomosis was made small, 4 to 7 millimeters long, without the excision of an ellipse from either vessel. After completing the upper caval-caval anastomosis, the lower caval-to-caval and portal-to-portal anastomoses were performed in anatomic position (Figs. 1c and 5b).

The third method of venous reconstruction resulted in normal venous pathways. The preliminary portacaval anastomosis was 8 to 10 millimeters in length and was used only to decompress the portal system (via the external polyethylene bypass) during the period of acute portal-caval occlusion as the liver was implanted. The portal-portal and lower caval-caval anastomoses were performed anatomically with No. 6-0 and No. 5-0 silk respectively (Fig. 1c). Before the abdomen was closed the portacaval shunt was taken down and the venotomies were closed with No. 6-0 arterial silk (Fig. 5c).

After completing the venous anastomoses, the aortic graft was placed in the right paravertebral gutter and attached with an end-to side anastomosis to the recipient dog's aorta, at a variable distance below the renal arteries (Fig. 1c). No. 6-0 arterial silk was used and a button of recipient aorta removed.

Internal biliary drainage was performed with a cholecystojejunostomy, below which was established a jejunojejunostomy (Fig. 1c). Both gastrointestinal anastomoses were performed with a two-layer catgut and silk technique. Splenectomy was carried out, and the abdomen was closed with multiple layers of fine silk.

POSTOPERATIVE CARE

One million units of aqueous penicillin were given on the morning of operation and twice daily during the entire postoperative period. As soon as the operation was completed, a large gastric tube was passed and kept in position until no longer tolerated. Frequent endotracheal aspirations or bronchoscopies were performed until the animal was fully awake.

During operation, 500 cubic centimeters of blood were generally given. During the first 8 to 10 postoperative hours, it was almost always necessary to give another 500 cubic centimeters, even to those dogs in which no bleeding could ever be demonstrated. Intravenous fluids were usually administered for the first 2 or 3 days, after which many dogs tolerated a low protein diet. If the animals stopped eating, intravenous or clysis therapy was reinstituted.

RESULTS

Tolerance of the devascularized liver to ischemia. The time between removal of the cooled donor liver and its revascularization in the recipient dog was 45 minutes, at the least, and in many instances 2 hours. After more than 2 hours, ischemic liver injury occurred which almost always precluded success by any of the described means of reconstruction. When blood flow was restored after ischemia of longer than 2 hours, the liver became tense and dark in color, with histologic evidence of intense congestion. Death followed in a few hours either from hemorrhagic gastroenteritis, as a result of hemorrhage from small capsular tears in the distended liver, or from acute hepatic failure.

In preparation of the donor liver, it was important that the liver be cool

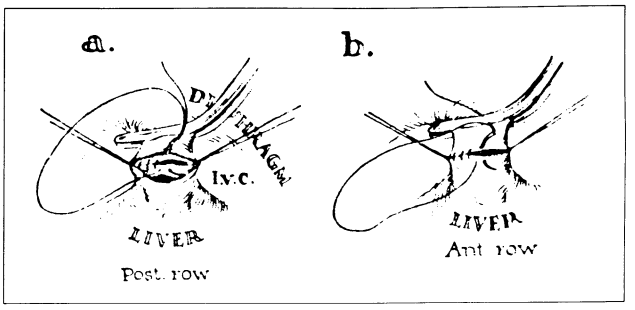

Fig. 4.— Technique of caval-caval anastomosis with posterior row being sutured from within.

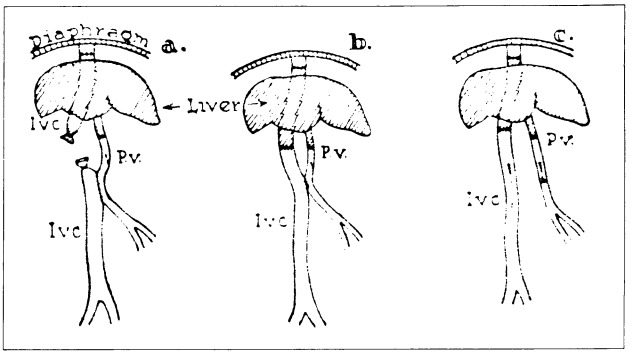

Fig. 5.— Methods of venous reconstruction. a, Reverse Eck fistula. b, Anatomic reconstruction with small portacaval shunt. c, Complete anatomic reconstruction with closure of preliminary portacaval shunt.

before any ischemia occurred. For this reason, total body cooling (25 to 30 degrees C.) was carried out in the donor dog before the abdomen was opened. In the event of accidental ischemia during dissection of the portal triad or aortic pedicle, before the liver was further cooled by perfusion, some protection was afforded.

After the liver had been perfused with cold Ringer's solution, its temperature was well maintained at approximately 10 to 16 degrees C. after removal. Shown in Figure 6 is a typical warming curve of the interior of a donor liver, in which the temperature rise was less than 2 degrees C. in 1-1/2 hours.

The value of the external bypass during liver transplantation. In performing the first 20 liver transplants, an external bypass was not used during the period of portacaval occlusion, since in the previous work it had been determined that short term occlusion in the presence of a portacaval shunt was well tolearted (29). Survivals of as long as 6 days were obtained with this technique, but in subsequent experiments the external bypass was routinely used for several reasons. The portacaval occlusion was often for as long as 2 hours, during the latter part of which profound hypotension usually developed. At autopsy, many of the dogs had cardiac lesions similar to the infarcts described by Tanturi after temporary portal occlusion. Finally, hemorrhagic gastroenteritis proved to be a common postoperative complication, and it was not possible to know if this was due to the acute portal hypertension during implantation or to splanchnic venous stasis which developed postoperatively.

With the external bypass, severe hypotension did not develop. Some fall in blood pressure occurred, but the mean pressure almost always remained above 100 millimeters of mercury. The bowel remained pink. Clotting of the siliconized tube did not generally occur during the bypass.

Acute effect of venous reconstruction with the reverse Eck fistula. Twenty-six hepatic transplants were performed by the method of the reverse Eck fistula (Fig. 5a). Only 4 of the 26 dogs survived for more than 4 days. The other 22 animals died at varying times up to 4 days, in 11 experiments as a result of the inability of the transplanted liver to transmit the required venous flow, with consequent hemorrhagic gastroenteritis. The other early deaths were caused by hemorrhage in 3 dogs, portal thrombosis in 3 dogs, atelectasis in 2 dogs, air embolus in 1 dog, and

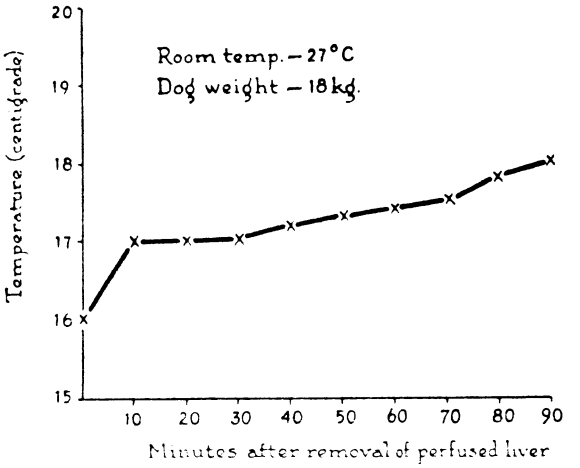

Fig. 6.— Rewarming curve of interior of donor liver exposed to room temperature for 1-1/2 hours.

unknown etiology in 2 dogs.

Most of the unsuccessful experiments followed a highly characteristic pattern. After revascularization, it became obvious that sequestration of blood was occurring in the liver which became swollen, tense, and extremely dark in color. Shortly after, large amounts of lymphatic fluid escaped from the surface of the liver capsule. The portal vein and inferior vena cava distended with pressures as high as 250 millimeters of saline. Superficial lacerations in the transplanted liver began to hemorrhage, and frequently the previously dry portal-portal or porta-caval anastomosis began to ooze, sometimes uncontrollably. Within a few minutes, the bowel and stomach lost their normal pink color and assumed a faintly cyanotic hue. Within a few hours, large quantities of fluid accumulated in the gut — at first clear and later blood-stained.

In such cases, a progressively increasing rate of blood transfusion was necessary to maintain the blood pressure. Bloody diarrhea developed, and the animals died in 5 to 15 hours, despite aggressive therapy. Histologic sections of the liver showed acute congestion and early disruption of hepatic cord architecture (Fig. 7a). Sections of the large and small intestine revealed marked congestion and interstitial edema of the entire wall (Fig. 8a and b).

In other experiments, the animals survived the immediate postoperative period, only to die 1 to 4 days postoperatively, usually from gastrointestinal hemorrhage. In these animals, there was histologic evidence of persistent hepatic congestion with distention and coalescence of sinusoids (Fig. 7b). Hepatic cell loss was apparent, especially in central areas. Associated slough of intestinal mucosa and inflammatory infiltration of intestinal villi occurred (Fig. 8c).

Four of the 26 liver transplants revascularized with the reverse Eck fistula survived longer than 4 days, or long enough for homograft rejection to be expected to occur. Maximum survival was 9 days.

Numerous complications characterized this group of animals. Formation of ascites was excessive, and dissection of the fluid through the incision with wound breakdown occurred in all long term survivals. Pneumonitis and thrombosis of the vascular anastomosis were also late causes of death. Only 1 of the long term survivors was able to eat.

Acute effect of anatomic venous reconstruction with addition of a portacaval shunt. Fifteen transplants were performed by means of anatomic reconstruction of the venous system with the addition of a small portacaval shunt (Fig. 5b). The portacaval anastomosis was deliberately constructed small — 4 to 7 millimeters — so that a definitely elevated portal pressure would be necessary in order for large flows to cross the shunt. In this way, it was hoped to decompress the liver and splanchnic circulation in the event of high portal pressures, without unnecessarily diverting portal blood from the liver. Fifteen livers were transplanted in this manner and 6 animals survived for more than 4 days. Nine dogs died in the early postoperative period: 2 of hemorrhage, 4 of atelectasis, 2 of hemor-

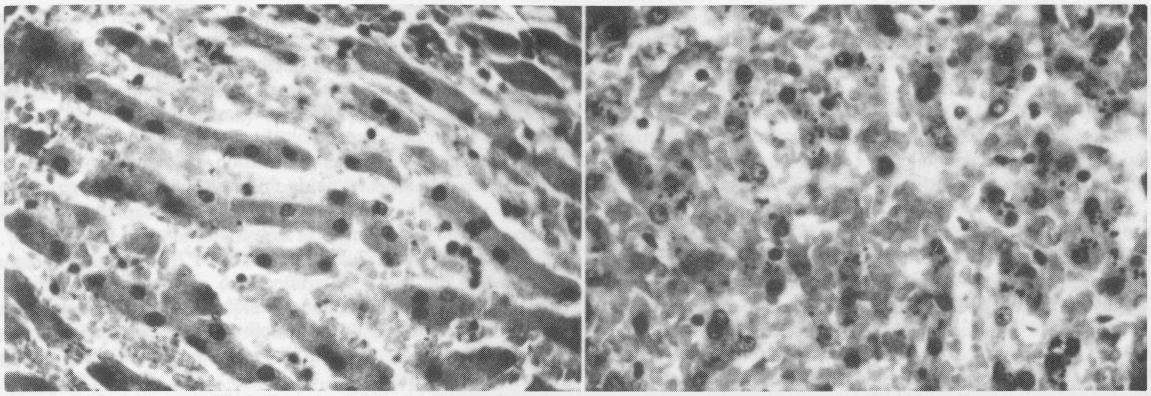

Fig. 7.—Histologic changes in hepatic outflow block. a, Liver after 10 hours, showing congestion and early loss of architecture with dilated sinusoids. b, Another liver at 36 hours showing persistent congestion and marked disorganization of hepatic architecture. X 214.

rhagic gastroenteritis, 1 as a result of portal thrombosis. An important contributory cause of death in many of these dogs was a distinctive syndrome consisting of intractable vomiting and retching which began immediately after operation and continued until the death of the dog. This was thought, but not proved, to be due to acute liver failure.

With this technique, the acute congestion of the liver and bowel described for the reverse Eck fistula was seen in only 2 experiments. Generally, the liver became soft and light in color after revascularization, and the bowel remained pink except in those cases in which the shunt was inadvertently made too small to be functional.

Despite the obvious disadvantage of reducing the blood flow to the liver, this technique of reconstruction proved to be the most versatile in one respect. It was possible to perform transplantation without closely matching the size of the donor and recipient animals. Donors were used which weighed as much as 8 to 10 kilograms less than the recipient animals, a disparity in size which precluded success in the other two methods because of the development of splanchnic and hepatic congestion.

Six of the 15 dogs with this type of venous reconstruction lived for more than 4 days. Only 2 animals were able to eat consistently. Maximum survival was 9 days. Although it is thought that homograft rejection occurred, a variety of other complications were found at autopsy which could have been independent causes of death.

Acute effect of anatomic venous reconstruction. Eleven dogs had reconstitution of the liver in an anatomic manner, and 8 survived for more than 4 days. The causes of early death were hemorrhagic gastroenteritis in 2 cases, and atelectasis in the third. When this technique was used, the acute bowel congestion described with reverse Eck fistula reconstruction was seldom encountered provided the weights of the donor and recipient dogs were closely matched. However, efforts to use significantly smaller donor than recipient animals resulted in acute bowel congestion and early death.

The clinical behavior of this group of dogs was the most satisfactory of all techniques tried. All 8 long term dogs resumed oral intake promptly, usually after 2 days, and continued to eat until shortly before death, which was presumably due to liver rejection. The average long term survival exceeded that obtained with either of the other methods, as did maximum survival which was 20-1/2 days.

Fig. 8.— Intestinal changes resulting from outflow hepatic block in dogs with reverse Eck fistula. a, Small intestine after 10 hours showing mucosal and submucosal congestion and submucosal edema. Note intact epithelium. X 11. b, Small intestine in another dog after 18 hours. There is congestion and loss of epithelium of distal portions of villi. X 44. c, Small intestine in a dog dying after 36 hours. Base of villi have partially intact epithelium, but distal villi are edematous and congested with marked slough of epithelial covering. X 44.

DISCUSSION

The unusual susceptibility of the liver to anoxia has undoubtedly deterred progress in hepatic transplantation. Welch and associates (9), who transplanted the whole organ to the pelvis of recipient dogs, pointed out that the transferred tissue could not survive if devascularized for more than 30 minutes under normothermic conditions. Disclosures of the protective effect of hypothermia on the ischemic liver by Raffuci, Bernhard, and Huggins and their associates have made transplantation studies more practical.

Despite the protection of hypothermia, undue prolongation of ischemia results in a characteristic liver injury in which the revascularized organ entraps large quantities of blood. The inability of blood to escape is probably due to constriction of small intraparenchymal hepatic veins as shown by Deysach and Thomas and Essex. These vessels have been demonstrated by Arey to have extraordinarily well developed muscular coats in the dog. Similar hepatic congestion, aptly termed "blue liver syndrome" or "outflow block," has been described in the dog in a variety of physiologic circumstances including: anaphylaxis and peptone shock by Mautner and Pick, Simonds, and Weil; temporary devascularization of the liver by Hines and Roncoroni and State and Lichtenstein; histamine injection by Mautner and Pick and Bauer and his associates; perfusion of the isolated liver by Makravarti and Tripod, Maegaith, and Kestens and his associates; endotoxin shock by MacLean and Weil; and hemorrhagic shock by Frank (6) and Wiggers and their associates.

With transplantation of the liver, the degree of ischemic injury is only one of the influences which contribute to the grave complication of outflow block. The most important secondary factor is the volume of venous blood which is transported to the liver. In the normal dog, it has been shown by Meyer and others that both the splanchnic and inferior caval venous return can be directed through the liver without harmful effects. But the anoxic transplanted liver apparently reacts to such augmented venous flow with outflow block. In contrast, the blue liver developed infrequently when venous inflow was normal or when a decompressing portacaval anastomosis was retained as a permanent feature of the reconstruction. The importance of regulating portal vein inflow in avoiding outflow block during isolated liver perfusion has also been commented upon by McDermott and associates (14), as well as numerous earlier workers.

Another factor contributing to the susceptibility to outflow block of the transplanted or the isolated perfused liver may be the state of denervation in both instances. The confusing literature on vasomotor control of the liver has been summarized by Child. The dominant autonomic influence appears to be sympathetic, with reduction of the size of the liver and increased outflow upon stimulation. Parasympathetic stimulation has resulted in less clear findings. Similarly, epinephrine solutions cause shrinkage of the liver with increased hepatic vein outflow, as shown by Bauer and his associates and by Maegaith, while acetylcholine has a less pronounced but reverse effect. From this information, it would seem quite possible that the denervated liver undergoes vasomotor alterations which promote stasis. Such a mechanism, in addition to contributing to outflow block, could explain the necessity for the large transfusions which are necessary postoperatively, even in the dogs which survive for long term study.

The sequence of events in the bowel following outflow block in the transplanted liver is of interest because of the resemblance of the pathologic changes of those seen in irreversible hemorrhagic shock. For many years, the liver has been suspected of playing an important role in the development of irreversibility. Numerous observers, including Selkurt (25), Wiggers, and Friedman and their associates, have reported increased hepatic vascular resistance after experimental hemorrhage, and it has been reasonable to believe that consequent splanchnic pooling and reduction of circulating blood volume would result. This concept was apparently strengthened by the demonstration of Fine and his associates (7) that viviperfusion of the liver during hemorrhage prevented the hemorrhagic gastroenteritis of irreversible shock. However, Fine's group (6) also demonstrated that decompression of the portal system with portacaval shunt to prevent impounding of blood in the splanchnic system did not prevent the intestinal lesions. Subsequently, attention has been focused on other than hepatic factors in irreversibility, and recently the intestine itself has been suggested as an important primary target organ in shock by Schweinburg and his associates, Selkurt, and Lillehei.

The assessment of the relative importance of the liver and that of bowel in determining irreversibility of shock is still not settled, although there is evidence that both play an important role. In the present study, an experimental situation was present in which only the liver was subjected to anoxia. The blood pressure generally was never below 100 before, during, or immediately after liver was revascularized. Yet, in the dogs developing outflow hepatic block, shock soon ensued which ultimately was intractable to further transfusion and which eventuated in fatal hemorrhagic gastroenteritis. In this situation, the damage inflicted upon the liver by the transplantation would seem to be solely responsible for the inception and development of irreversible shock.

SUMMARY

The homologous canine liver has been transplanted to recipient animals in which total hepatectomy and splenectomy have been performed. The longest survival after placement of the liver homograft was 20-1/2 days.

Protection from hepatic ischemia for as long as 2 hours was obtained by cooling the donor liver to 10 to 20 degrees C. The arterial supply was restored through a hepatic artery-aortic pedicle which was removed in continuity with the liver and anastomosed to the descending aorta of the recipient. Internal biliary drainage was established.

The volume of venous flow transmitted to the transplanted liver has been shown to be an important determinant of success. When this was excessive, as when both the portal and inferior caval flows were directed through the liver, hepatic outflow block usually developed with consequent fatal congestion of the hepatic and splanchnic beds. When the portal flow was normal or reduced, outflow block rarely occurred.

An attempt has been made to relate the development of outflow block as it occurred in the transplanted liver to other circumstances, including hemorrhagic shock, in which similar phenomena have been observed.

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Studies on the rejection of the transplanted homologous dog liver

Surgery, Gynecology & Obstetrics, 112: 135-44, 1961

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In a contemporary report, the technical problems and complications encountered with homotransplantation of the dog liver were described (15). As occurs with other vascularized homografts, the liver appeared to be rejected by the host after a characteristic time interval, usually 6 to 10 days.

The present study is concerned with an analysis of events, both in the homografted liver and the host, in 18 dogs which survived 4 or more days after liver transplantation, long enough presumably for homograft rejection to occur. This included postmortem and tissue studies, as well as chemical and hematologic determinations during life. Strong histologic evidence has been obtained of widespread participation of the host reticuloendothelial system in the rejection, quite comparable to that seen after bacterial and foreign protein immunization.

METHODS

The techniques used for liver transplantation have been previously described (15). The homograft was positioned in the liver fossa, after removal of the recipient dog's liver, and splenectomy was performed. Arterialization and internal biliary drainage were carried out with a uniform technique in all 18 experiments. Venous pathways were reconstructed with three variations: (a) anatomically, 8 cases; (b) anatomically with the addition of a small portacaval shunt, 6 cases; and (c) by diversion of both the splanchnic and vena caval flows through the liver, 4 cases. Although the method used was profoundly influential in determining early mortality, the type of venous connection was not an important factor in most of parameters analyzed in the present study, and the results apply to all dogs, unless otherwise stated.

Adult mongrel dogs were used. The donor and recipient were always chosen for obviously different color and general appearance. In about one-half the experiments, the donor and recipient were different sexes. Blood studies were obtained preoperatively and every 2, 3, or 4 days thereafter, and the removed blood was replaced with immediate transfusion. In a few animals, transfusions were also given for the treatment of late gastrointestinal hemorrhage. All chemical studies were standard determinations, made in a clinical laboratory. Autopsies were performed promptly, usually within an hour and never longer than 8 hours after death, and the specimens fixed in formalin. Hematoxylin-eosin stains were always used, and in some cases additional tissue stains were employed.

RESULTS

Survival. Survival times in 18 dogs are shown in Figure 5. Ninety per cent of the deaths occurred between the fifth and the tenth days. The longest survival was 20-1/2 days. Although many other factors contributed to death, evidence will be presented that graft rejection played an important role in most cases.

Clinical behavior. The dogs with the most sastisfactory course were those with anatomic venous reconstruction. They were usually able to eat after the second postoperative day. Diet generally consisted of brown sugar water and bread, but some of the dogs were hungry for and allowed to eat meat. Although the dietary intake of dogs with other than anatomic venous connections was poor, physical activity of the different groups of animals was frequently normal for the first 4 or 5 days.

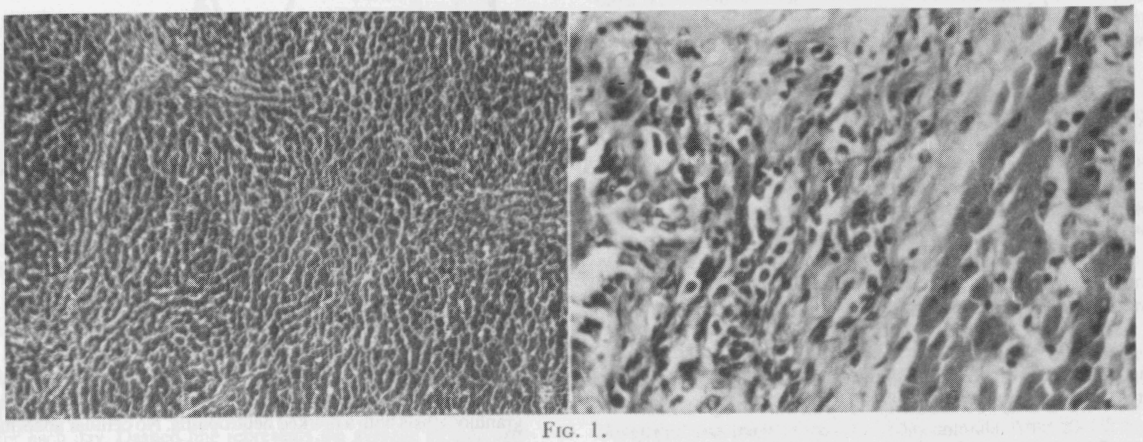
On the sixth or seventh day, jaundice developed in every experiment, usually within 12 to 24 hours after it was noted that the urine had become dark. Some of the dogs continued to eat, but usually dietary intake was sharply reduced. Jaundice was followed by death in 2 to 4 days in all but 1 animal in which the jaundice receded after the eleventh day. Terminally, pallor of the gums was often seen, and vomiting was common in both feeding and fasting dogs.

The clinical behavior of the longest survivor (No. 65) requires separate comment. Jaundice developed in this animal on the sixth day, and the dog appeared to be critically ill for 4 days. He continued to eat, however, and after the eleventh day improved with continued clinical and chemical regression of the jaundice (Fig. 6) until death after 20-1/2 days. This was the only unequivocal instance of improvement after signs of liver malfunction had occurred.

Rectal temperatures and femoral pulse rates were taken twice a day. All animals had low grade fever during most or all of the survival period. This was seen by the first day after operation and persisted without spikes (Fig. 7). There was no deviation from this pattern during the presumed rejection period. Correspondingly, pulse rates were not subject to wide variation after the immediate postoperative tachycardia had subsided (Fig. 7).

Most of the dogs lost about .25 kilogram in weight per day after operation. In some cases, the tissue loss was undoubtedly greater, since the development of ascites was often prominent.

Blood chemistry. Fasting blood sugars were obtained before and after



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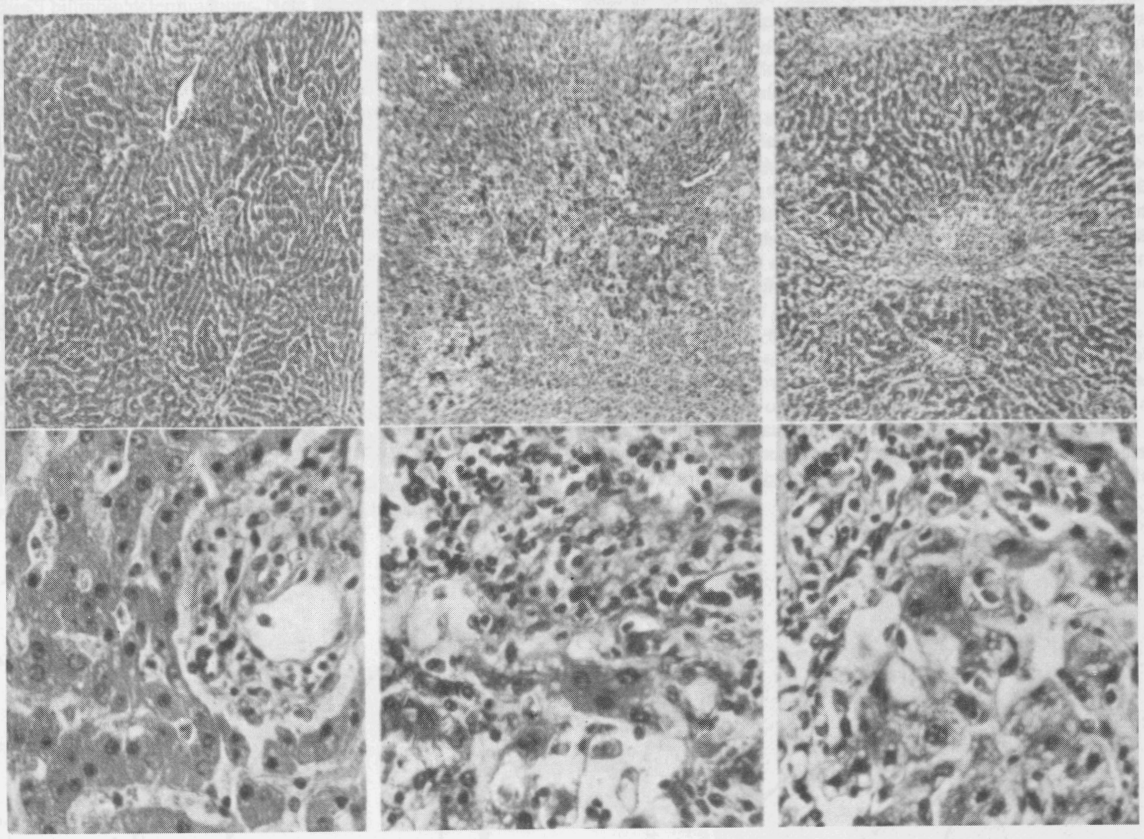

Appearance of liver homografts at varying times after transplantation.
Fig. 1.— Five days: left, X 42; right, X 263.
Fig. 2.— Nine days: above, X 42; below X 263.
Fig. 3.— Ten days: above, X 42; below X 263.
Fig. 4.— Twenty and one-half days: above, X 42; below, X 263.

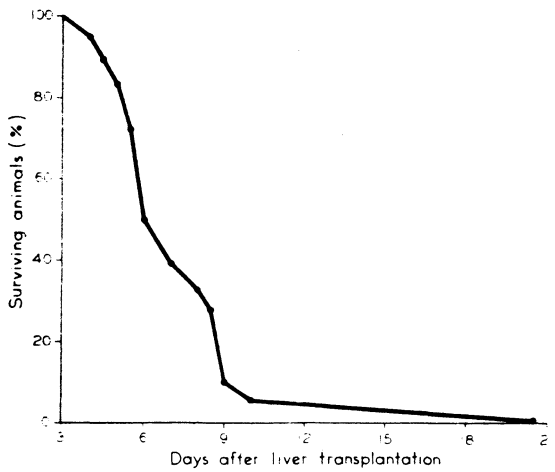

Fig. 5.— Survival in dogs living more than 4 days. Note high mortality between fifth and tenth day.

operation. Samples were taken in the morning, after an 8 to 12 hour withdrawal from food or intravenous fluids. Initially, blood sugars remained at a normal level. However, at varying times, from 4 days on, blood sugars as low as 15 to 25 milligrams per cent were observed (Fig. 8). With longer suvivals, a definite correlation was evident between survival time and degree of hypoglycemia (Fig. 8). In most dogs, a dropping blood sugar was a preterminal event, but in the longest survival the blood sugar fall reversed itself as other liver chemistries improved (Fig. 6).

The time of development of chemical jaundice was very definite. By the fifth day, only 1 dog had a rise in bilirubin, but by the sixth day all tested dogs had become jaundiced (Fig. 9). The increase in the direct bilirubin fraction was less but paralleled the total rise (Fig. 9). Comparable rises were seen with alkaline phosphatase, starting on the fourth or fifth day (Fig. 10).

Total proteins were not predictably altered by transplantation. An example is shown in Figure 11 with determinations from the 20-1/2 day survivor, in which major shifts in either the total or the albumin-globulin fraction did not occur. Thymol turbidity tended to increase, but inconstantly. Blood cholesterol rose (Fig. 12), remained stable, or fell with about equal frequency. Blood urea nitrogen remained normal in all dogs until terminally, when in 3 of 11 animals studied a sharp rise occurred (Fig. 12).

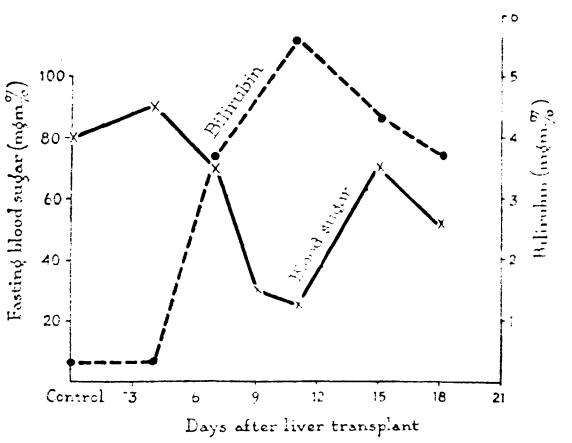

Fig. 6.— Blood sugars and bilirubin in 20-1/2 day survivor (No. 65). Note improvement in chemistries after eleventh day.

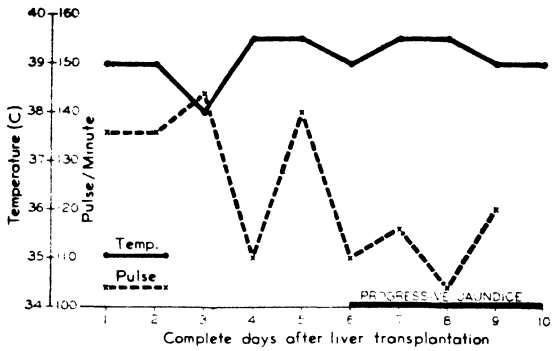

Fig. 7.— Fever and pulse of dog 76. Animal had no complications. Cause of death probably homograft rejection.

Hematologic studies. Complete blood counts were obtained before and at varying times after operation. Because of transfusions, analysis of erythroid values was not meaningful. All animals, except 1, had rises in the white count, sometimes as high as 40,000. The rise was specifically due to granulocytosis with a marked neutrophilia. No cellular abnormalities were noted in the peripheral blood, and in particular plasma cells were not seen.

Urine studies. All but 1 of the dogs excreted adequate urine until just before death. In 3 of 8 animals tested, albuminuria developed after 2 to 4 days and persisted. Analysis of bile products disclosed differences in urines collected before and after 4 complete postoperative days. In 8 specimens collected from different animals before the fourth day, bile was detected in only 2. After the fourth day, 13 of 14 urines contained bile. Before the fourth day, half of the specimens contained urobilinogen (more than 1.0 Ehrlich unit), and after the fourth day urobilinogen was detected in only 5 of 14 samples.

Gross pathologic findings. The homografted liver had characteristic gross findings. It was usually larger than normal with a tan appearance and firm consistency. Although it had no other attachments than the various anastomoses, there were no instances of dislocation of the organ from the liver fossa. On section, the tissue cut with a firm and gritty sensation. The transected surface had a nutmeg appearance, such as seen with chronic heart failure. In every case, the cholecystojejunostomy and enteroenterostomy were intact.

Thrombosis of a vascular anastomosis occurred in 4 of the 18 experiments, twice in the aortic graft and once each in the portal vein and inferior vena cava. In 1 dog, the aortic suture line disrupted after 5 days. In

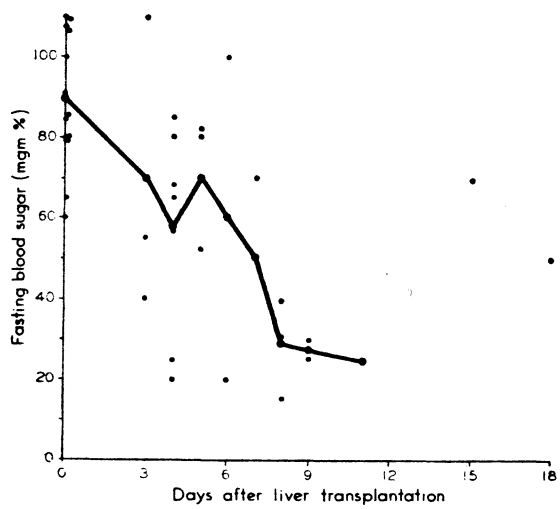

Fig. 8.— Fasting blood sugars (dots) in all dogs not treated with intravenous glucose. Solid line connects average values for each day. Note that severe hypoglycemia sometimes developed as early as the fourth day.

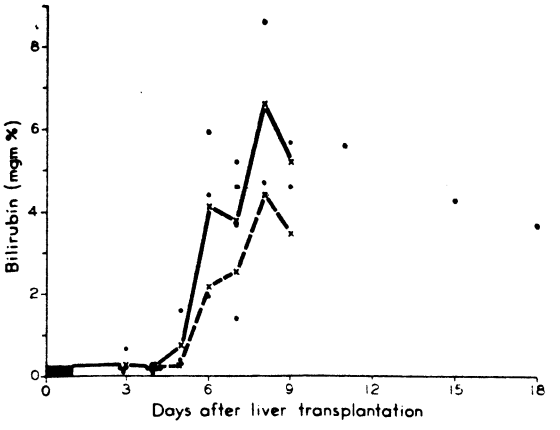

Fig. 9.—Scattergram showing pattern of onset of jaundice. Dots are individual total bilirubin determinations. Solid line connects average total bilirubin for each day. Dashed line represents the average of the corresponding direct bilirubin determinations. Note absence of chemical jaundice until sixth day.

the 10-1/2 day survivor, aortic rupture may have occurred, but it was not possible to be certain of this at autopsy.

The abdomen contained from 25 to more than 1,000 cubic centimeters of bloodstained fluid. Ascites was most prominent in animals in which both caval and portal flow had been transmitted through the liver. Wound infection, a complication in 5 of the 18 dogs, occurred in animals with severe ascites in which the fluid dissected through the incision. Significant pleural effusion occurred in only 1 dog.

Some of the host organs regularly exhibited gross abnormalities. The lungs invariably had a peculiar firm leathery consistency. In addition, some of the animals had atelectasis, pneumonitis, or pulmonary edema. In 6 of the hearts there was focal epicardial necrosis, the lesions being confined to the right ventricle. Usually, superficial sloughing or ulceration was found in some portion of the gastrointestinal tract, most prominently in the duodenum. In 6 of the 18 dogs, multiple shallow duodenal ulcers were encountered, and in 2 more, duodenal ulcers were amost perforated. The kidneys and adrenal glands were normal. Host lymph nodes in the mesentery, mediastinum, and elsewhere were generally enlarged.

Microscopic findings. As noted previously (15), hepatic architecture was well preserved during the first few days after operation, unless hepatic congestion or other complications of revascularization occurred. After 4 days, alterations developed in all liver homografts which were usually related quantitatively to the period of survival. At first, hepatic cell loss was absent or minimal (Fig. 1). However, even in dogs surviving only 4 or 5 days, a mononuclear infiltrate of plasma cells and lymphocytes appeared. These cells were most prominent in the periportal areas but were also seen diffusely throughout the parenchyma (Fig. 1). Endothelial and adventitial proliferation of small arteries was often seen.

After 6 days, undisturbed hepatic architecture was found in only 1 homograft (dog 20), from a 9 day survivor. The only striking change in this exception consisted of a periportal and diffuse mononuclear infiltrate (Fig. 2), consisting of plasma cells and lymphocytes. In all other experiments, there was more or less extensive hepatic cell loss, sometimes most prominently around the central vein (Fig. 4). In other instances, the parenchymal loss was general and so extensive that residual liver cells could be identified only with difficulty (Fig. 3). The liver was in these cases almost completely replaced with a massive mononuclear infiltrate. Congestion of the homograft completed the picture, often with extravasation of red cells into the structureless tissue (Fig. 3). In a few animals, necrotizing arteriolitis was seen. The presence of bile pigment in the hepatic cells was an inconstant finding (Fig. 4).

The longest survival had homograft changes which were less well developed than many of the 6 to 10 day animals. After 20-1/2 days, a semblance of organized structure remained (Fig. 4). Parenchymal loss was chiefly around the central veins. The diffuse and periportal mononuclear infiltrate, comprised chiefly of plasma cells (Fig. 4), was comparable to that found in the shorter survivals. Many of the intrahepatic bile ducts were denuded of epithelium. Connective tissue stains did not reveal the presence of fibrosis. Reticulum stains showed good preservation of the reticular pattern.

In 7 experiments, enlarged lymph nodes from the posterior mediastinum were studied. In all cases, there was more or less cortical thinning with a decreased number of follicles (Fig. 13) which contained principally lymphocytes and monocytes. Lymph pulp was increased with a large number of plasma cells in the medullary cords. Supporting tissue around the nodes was infiltrated with plasma cells.

Aggregates of plasma cells and other mononuclear cells were found in all host kidneys. These were principally in the cortex, usually in a periglomerular or perivascular position (Fig. 14a). There was endothelial and adventitial proliferation of the small vessels, often with edema. In one-

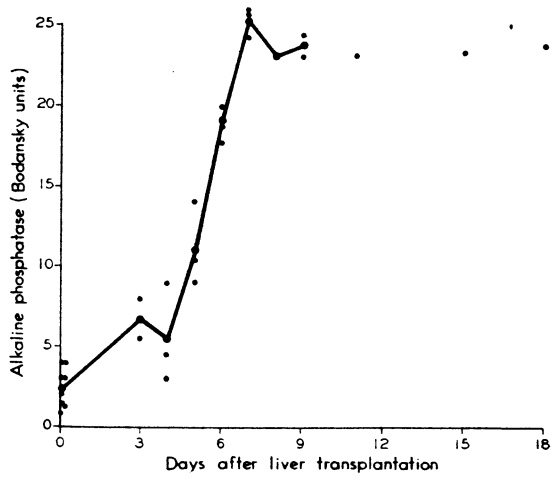

Fig. 10.— Alkaline phosphatase levels in 8 dogs. Note marked rise after fourth day.

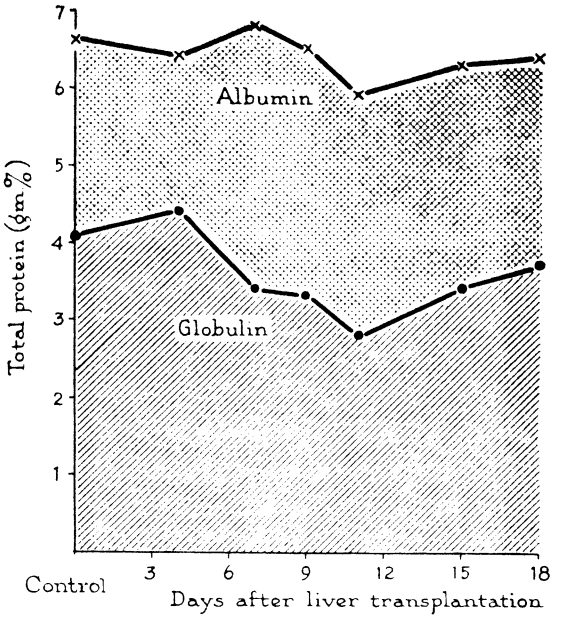

Fig. 11.— Total protein and albumin/globulin fractions in dog 65.

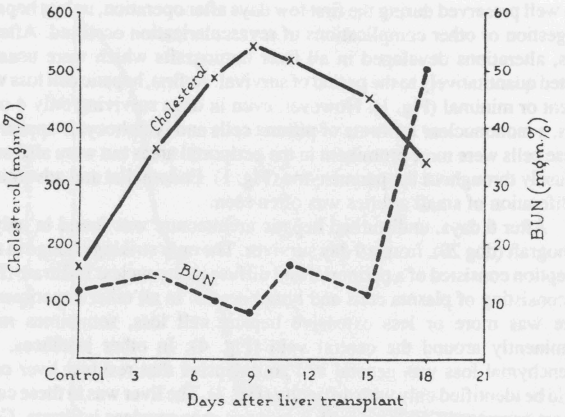

Fig. 12.— Cholesterol and blood urea nitrogen in dog 65. Terminal uremia was seen in minority of animals.

third of the cases, a more diffuse plasma cell and mononuclear response was also found throughout the kidney. In addition, a moderate to marked plasma cell involvement was noted in the perirenal (Fig. 14b) and periadrenal tissues.

Pulmonary abnormalities included passive congestion, pulmonary edema, atelectasis, and pneumonitis. However, in 15 of 18 experiments, a specific abnormality was noted. There was thickening of the pulmonary alveolar wall, apparently due to endothelial and histiocytic proliferation in the septa (Fig. 15). A light plasma cell infiltrate was usually present in the parenchyma with focal accumulations around small blood vessels. Some vessels had changes similar to those described in the kidney. Multinucleated giant cells were very numerous (Fig. 15).

Bone marrow studies were made. In every experiment, increased numbers of plasma cells and lymphocytes were found. In some cases, these infiltrates were moderate, but in other dogs (Fig. 16) the relative and absolute increases in plasma cells and lymphocytes were striking and extensive.

Skeletal muscle from the foreleg was studied in all 18 dogs. No diffuse or focal plasma cell response was found in any case.

Findings in the heart and gastrointestinal tract were related partially to traumatic artefact. Focal myocardial infarcts often occur as a direct result of operation (15). In the later dogs, organizing infarcts or mononuclear infiltrates were seen in all experiments. In the gastrointestinal tract, hyperemic sloughing and gastritis or enteritis were seen in various experiments. A diffuse plasma cell and lymphocyte infiltration was always present, often to a marked degree.

Causes of death. The precise mechanism of death was often difficult to determine, not only because the findings with homograft rejection are not specific, but because of the frequent coexistence of serious postoperative complications. In 5 dogs, rejection appeared by exclusion of other factors to be the sole reason for death. In all dogs surviving more than 6 days, it was thought that rejection of the homograft was in process at the time of death, in view of the histologic and chemical evidence of hepatic deterioration.

Controls. Three types of controls were available for assessing the role of operative trauma or surgical artefact in the foregoing results. The first of these involved analysis of dogs which died after liver transplantation as the direct result of operative trauma, in from a few hours to a day or so after surgery. In these animals, hepatic architecture was preserved, except when "outflow block" had occurred. There was no infiltrate in the livers. Fresh focal infarcts were found in the myocardium. The histologic changes in the bone marrow, kidney, lymph nodes, and lung described for longer surviving animals were not present.

In 4 control dogs, cholecystojejunostomy with enteroenterostomy was performed after ligation of the common duct. The dogs were sacrificed at 5, 13, 14, and 15 days. Histologic studies of the liver were either normal or disclosed a minimal periportal polymorphonuclear infiltrate. In no case did bilirubin or alkaline phosphatase rise, nor were other chemistries changed. All other organs were normal.

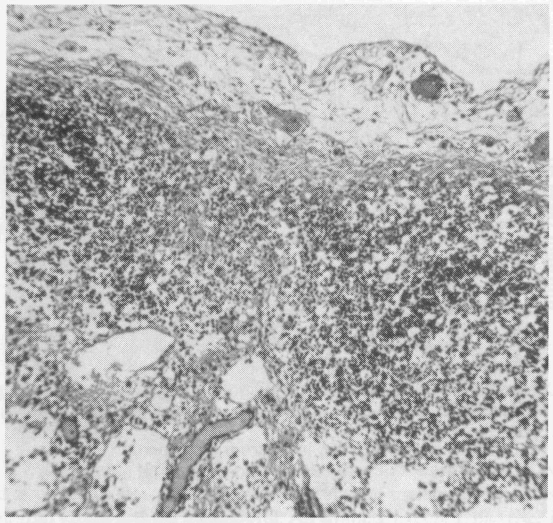

Fig. 13.— Mediastinal lymph node from dog 65. X 82. Note cortical thinning.

Finally, in 4 more dogs, sham operation was performed involving autografting the liver. The steps employed were essentially the same as with homograft experiments, except that the dog's own liver was returned to the liver fossa. Postoperative chemical studies did not show the abnormalities described after homografting. The dogs did not become jaundiced or ill. The animals were sacrificed after 7 to 15 days. Focal myocardial infarcts or infiltrations were present in all 4 animals. Liver architecture was completely preserved, and mononuclear infiltrates were not present. The histologic abnormalities noted in the foregoing in the bone marrow, lung, kidney, and lymph nodes were not found.

DISCUSSION

Dempster, Moore and associates (12, 13), and other students of whole organ transplantation have recognized the need for delineating changes ascribable to the presence of a homograft from those alterations which result from surgical trauma. Some nonspecific artifacts were found in the present studies. For example, the control studies indicated that the lesions in the myocardium were explicable by the trauma of massive surgery. However, the changes in the chemistries and the consistently noted pathologic findings in the liver, bone marrow, kidneys, lungs, lymph nodes, and possibly gastrointestinal tract seem clearly related to the placement of the homograft.

The metabolic studies provided a means of following the repudiation of the liver graft by the host animal. It has been shown by Mann and subsequent observers that dogs will die in 1 or 2 hours after hepatectomy if continuous intravenous glucose is not provided. Under the same conditions, continued survival after replacement of the recipient's liver with a homograft was incontrovertible proof of hepatic graft function. Function was complete initially, but measurable deterioration began on the fourth to sixth day, with the onset of jaundice, the rise in alkaline phosphatase, and the tendency toward hypoglycemia. The high direct blood bilirubin component, in conjunction with the elevated serum alkaline phosphatase and urinary bile, suggested obstructive jaundice. Since extrahepatic obstruction was ruled out by control studies, it was concluded that obstruction was diffuse and intrahepatic. Such an occurrence was not hard to envision in view of the distorted, firm, and enlarged liver found at autopsy. In previous studies on liver homografts transplanted into the pelvis, Welch and his associates (7) noted the cessation of bile flow after 4 days. From the present study, it appears that such an impaired liver can continue at a reduced level of efficiency to sustain life for many additional days.

Comparisons are evident between the present results and those obtained by Simonsen and Dempster with homotransplantation of the kidney. Survival times were somewhat longer with the liver, since mean survival with kidneys was 4 days. Kidney transplants exhibit an immediate gradual functional deterioration with a rising blood urea nitrogen from the

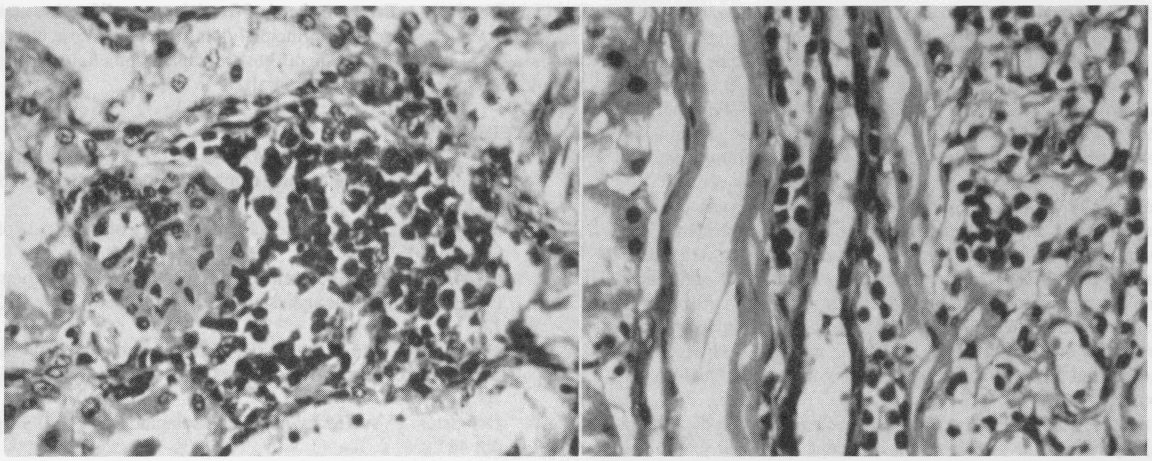

Fig. 14.— Host kidney from dog 76 showing: a, perivascular mononuclear aggregate of cells and vascular changes, X 232; b, cellular response in perirenal tissue, X 232. Aggregates were mostly plasma cells.

first day. The liver appeared to function normally for about 4 days, but its subsequent deterioration was similarly gradual. Evidence was presented by Dempster that the terminal event in the kidney was small vessel spasm with consequent inability of the organ to transmit blood, a general concept of rejection that has been strengthened by Edgerton's observations with skin grafts. Conceivably, a similar mechanism in the liver accounted, by means of splanchnic pooling, for the late gastrointestinal hemorrhages and mucosal sloughing seen in some dogs in the present study. At autopsy, both the kidney and liver homografts were abnormally large, pale, and firm.

Histologic findings were also comparable in many respects. In the kidney, cortical changes were dominant with early perivascular and periglomerular infiltrates, and later with edema, hemorrhage, more marked cellular infiltration, and structural disorganization. In the liver, aggregates of plasma cells and lymphocytes centered principally around the intraparenchymal portal triads, but the major parenchymal loss was frequently around the central veins. The ultimate degree of architectural loss was generally greater in the livers.

The precise mechanism of homograft rejection is not known. As a result of the penetrating analyses of Medawar (10, 11), the theory of acquired immunity has become a widely accepted explanation. The theory holds that a homograft acts as an antigen to evoke an antibody response from the host which causes rejection of the graft and confers a permanent immunity to all tissues from the same donor. The interval between placement and rejection of the graft is accounted for as the time necessary for the antigenic substance to get to and mobilize a response from the antibody-forming centers. Algire has shown that invasion of the graft with host lymphocytes is necessary for rejection. The principal evidence that rejection is due to an acquired host immunity was the demonstration by Medawar (10) that a second graft from the same donor is rejected in an accelerated fashion.

In the present study, the fate of the liver homograft has been shown to be comparable to other transplanted organs or free grafts in that it is ultimately rejected by the host. The converse, namely an effort of the graft's antibody mechanism to kill the host, has been the subject of lively interest since Simonsen and Dempster suggested the possibility with kidney transplants. Both Billingham and Brent's "runt disease" and Trentin's "secondary homologous disease" are thought to result from the attack of mature homografts upon fetal and irradiated hosts respectively. In the case of the liver, which has been shown by Berg and others to constitute well over half, and perhaps more than 90 per cent, of the splenectomized dog's reticuloendothelial system, any reaction against the host would be expected to be magnified.

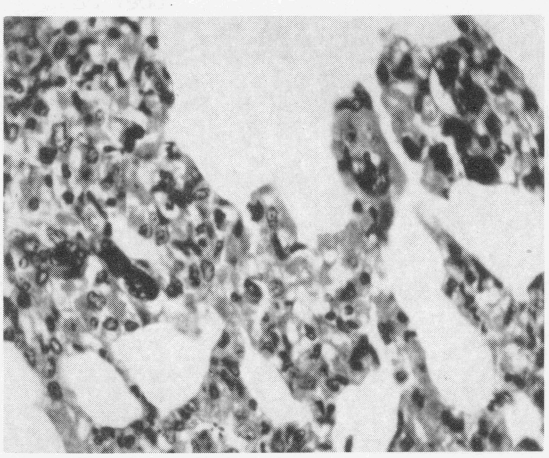

Fig. 15.— Host lung from dog 68 showing proliferative process in alveolar septa, and giant cells. X 225.

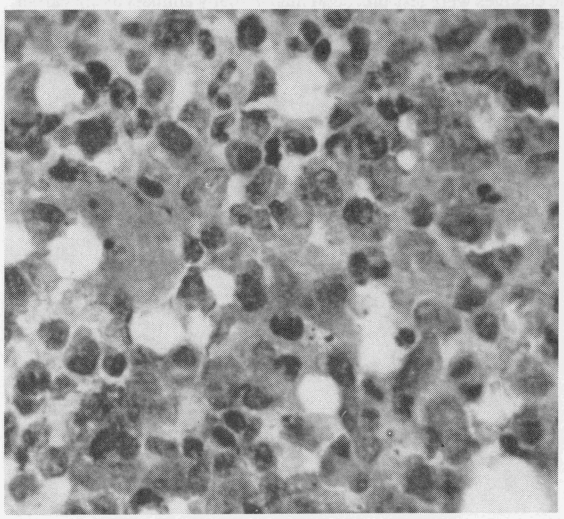

Fig. 16.— Bone marrow in dog 65, after 20-1/2 days. X 392. Note large numbers of plasma cells.

From a functional viewpoint, there was no consistent evidence that any of the host organ systems were seriously challenged by graft initiated cellular or humoral antibodies. Renal and hematopoietic functions were most reliably monitored. Urinary output was generally good, and rises in blood urea nitrogen occurred terminally in only 3 of 18 dogs. Postoperative granulocytic response, presumably of host bone marrow origin, was adequate and sometimes prodigious. Except for complications related to surgery, cardiac or pulmonary system deterioration did not occur.

Histologically, however, host organs known to possess reticuloendothelial elements were universally infiltrated with lymphocytes and especially plasma cells in the same general manner as the graft itself. In addition, active proliferation of fixed mesenchymal tissue in and around blood vessels was prominent, a finding which is also common in a rejecting graft. It would be tempting to regard these findings as evidence of a graft attack on recipient organs, especially since such multiorgan changes have not been noted after placement of smaller and less immunologically active homografts.

Admittedly, no definite conclusion can be reached, but certain evidence at the present time is against this concept. As noted previously, function of the host organ systems was not impaired. Secondly, if mononuclear cells were originating and migrating from the homograft to host tissues, one would expect a ubiquitous distribution. Instead, such cells were found only in those tissues which were themselves capable of a reticuloendothelial response. The recipient skeletal muscle, for example, had no infiltrate in any of the 18 animals. Finally, it has been shown by Bjornboe that prolonged and intense stimulation with various bacterial or protein antigens will cause a universal reticuloendothelial response, astonishingly similar to that described in the present study, and Kojima has shown the same thing with a pure lipid antigen. Consequently, at present it seems most reasonable to believe that the histologic changes in host organs predominantly represent an exhuberant response to the massive antigenic stimulation of the liver homograft.

An alternative possiblity is that the widespread alterations comprise a composite picture in which both graft versus host and host versus graft reactions have played a significant role. Studies in which either the liver graft or the host are rendered immunologically defenseless by irradiation should eventually clarify the issue.

SUMMARY

Dogs in which livers have been replaced with hepatic homografts usually die in 5 to 10 days. Liver metabolism is not detectably abnormal at first, but gradual deterioration of function commences on the fourth or fifth day.

There was histologic evidence of rejection in all dogs dying after 4 days. This ranged from minimal mononuclear infiltration to almost complete destruction of parenchyma. In the longest survivor, 20-1/2 days, histologic changes were less profound than in many animals dying earlier.

Widespread histologic changes were found in the host reticuloendothelial system, involving the bone marrow, kidneys, lungs, lymph nodes, and other tissues. These consisted of fixed tissue proliferation and infiltration of mononuclear cells, principally plasma cells. These changes were thought to be due to a general host reticuloendothelial response to the antigenic stimulus of the homograft.

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The same principles used for orthotopic liver transplantation were extended to the transplantation of all of the abdominal viscera as originally reported in abstract at the American College of Surgeons.* The perioperative mortality was 85%. The liver as part of an organ cluster was not rejected as vigorously as after liver transplantation alone, possibly because of the large antigen mass of the complex graft. Although graft-versus-host reactions were not well understood in 1960, these were recognized for the first time to have been caused by a solid organ graft. Twenty-seven years later, almost exactly the same operation was performed successfully in a child with a "short gut" syndrome and hepatic failure.

* Starzl TE and Kaupp HA Jr: Mass homotransplantation of abdominal organs in dogs. *Surgical Forum* 11: 28-30, 1960

Homotransplantation of multiple visceral organs

American Journal of Surgery, 103: 219-29, 1962

Thomas E. Starzl, Harry A. Kaupp, Jr., Donald R. Brock, George W. Butz, Jr. and James W. Linman

Recently, considerable information has been obtained on the behavior of liver homografts, by Moore et al. [8] and in our own laboratory [10, 11]. The large and immunologically active liver homografts were rejected in roughly the same time sequence as smaller and less complex tissues. In our studies, the manner of rejection differed from the usual situation in that widespread changes were evoked in the host reticuloendothelial system, involving the lungs, kidneys, lymph nodes, bone marrow and other organs. The functional response of the transplanted liver was substantially the same, both in Moore's studies [8] and in our own [11]. In all our animals, jaundice developed by the fifth day, and the animals died from one to sixteen days later.

In the present study, other abdominal viscera were added to the liver, to constitute a relatively enormous multiorgan graft consisting of the liver, spleen, pancreas, omentum, stomach, small bowel and colon. The behavior of the liver as a constituent of the multivisceral graft was compared with that previously studied with homotransplantation of the liver alone. Since the graft contains the major portion of the reticuloendothelial system of the body, particular attention was paid to the possiblity of a graft versus host reaction.

METHODS

Adult healthy dogs from 10 to 20 kg. in weight were used in thirty-eight transplantation experiments. The animals were dewormed, passively immunized against distemper, and prepared for surgery with a two or three day bowel preparation, using cathartics and 2 gm. of neomycin sulphate per day. All recipient dogs were females, and most donors were males. The donor dogs were 1 to 5 kg. lighter than the recipients. The animals were anesthetized with 25 to 30 mg. sodium pentobarbital and placed on respirators. Arterial pressures were monitored continuously during and after operation.

Blood chemical and hematologic studies were made before and at intervals after surgery. After death, complete autopsies were promptly performed and the tissues prepared for histologic study.

TECHNIC OF OPERATION

Preparation of Donor Tissues. Using two surgical teams, the operation was started in donor and recipient dogs at the same time. The donor animal was first cooled to 28° to 30° C. by immersion in an ice bath. The abdomen was opened and the entire abdominal aorta mobilized, ligating and dividing all branches, except the superior mesenteric artery and coeliac axis. (Fig. 1.) Mesenteric and other posterior parietal connec-

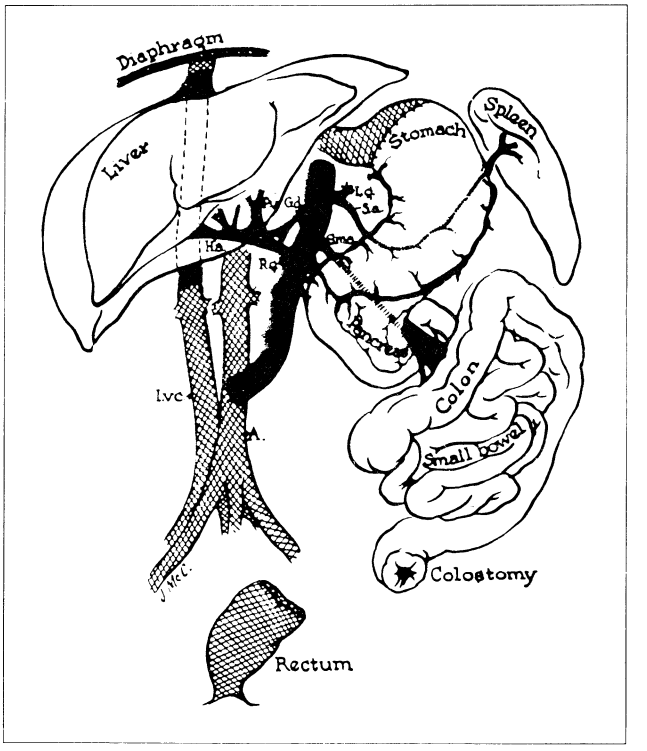


Fig. 1.— Schematic view of the transplanted tissues and their anatomic relation to the host. The grafted tissues are not shaded.

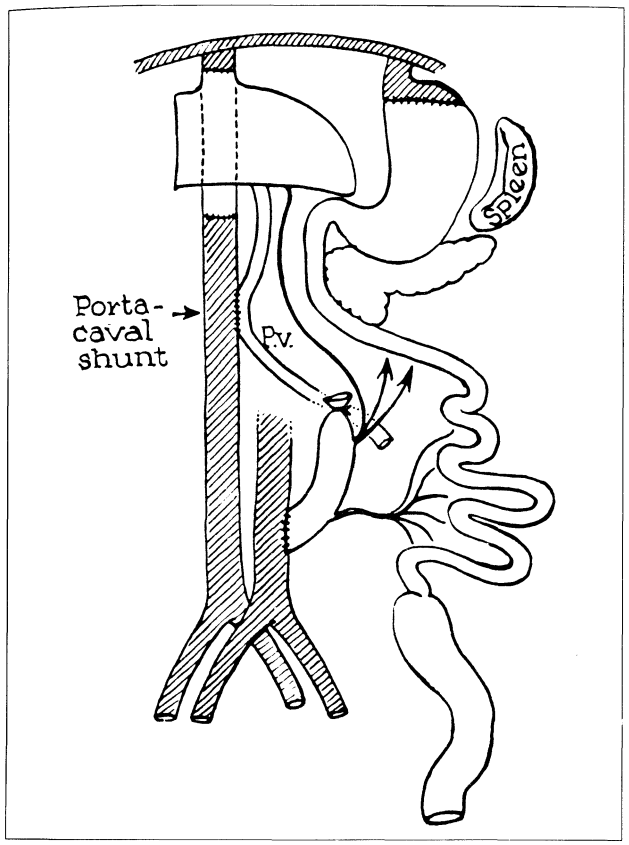


Fig. 2.— Addition of portacaval shunt to operation depicted in Figure 1.

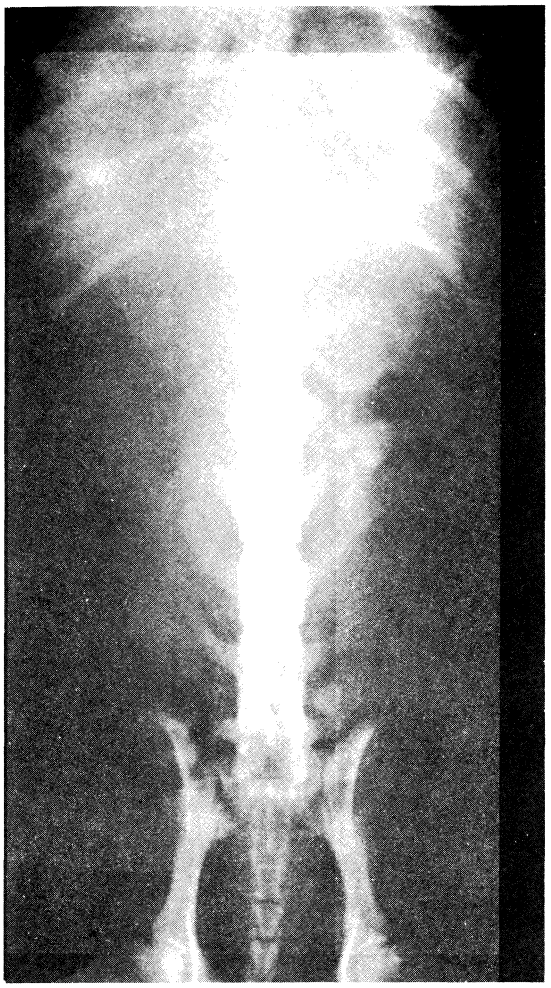


Fig. 3.— Abdominal roentgenogram of Dog. No. 18 on the sixth postoperative day. Dog had been on oral intake for four days.

tions, including the afferent fibers to the coeliac and superior mesenteric ganglia, were severed. The stomach was transected at the esophagogastric junction (Fig. 1) and closed in two layers. The colon was transected in the descending or sigmoid portions. A No. 17 gauge needle was inserted into the portal vein, and the liver was perfused with 1,000 cc. iced lactated Ringer's solution. During the last half of the perfusion, the dog was bled to death. The vena cava was then transected above and below the liver. The aorta was cut free well above the coeliac axis. The specimen was immediately removed and immersed in a bath of iced lactated Ringer's solution for at least three minutes.

Procedure in the Recipient Dog. While the homograft was being prepared, the recipient animal was opened with a long midline abdominal incision. The rectum was mobilized, transected and closed in two layers within 1 or 2 cm. of the anus. (Fig. 1.) The visceral attachments to the posterior parietes were severed, and the coeliac axis and superior mesenteric artery were ligated and divided. The stomach was divided leaving a small cuff at the diaphragm for subsequent anastomosis to the donor stomach. (Fig. 1.) The femorojugular venous bypass described by Kaupp et al. [4] was inserted, and the vena cava was grasped with Potts clamps and severed above and below the liver. The liver, stomach, pancreas, spleen, small bowel, colon and omentum were then removed *en bloc*.

The donor organs were removed from the ice bath, positioned and revascularized. The vena cava was anastomosed above and below the liver (Fig. 1), following which, the occluding clamps were released. The aortic homograft was attached to the abdominal aorta by an end to side anastomosis, and the specimen was arterialized. (Fig. 1.)

In five of the thirty-eight dogs, a portacaval anastomosis was also constructed between the recipient vena cava and the donor portal vein. (Fig. 2.) Gastrointestinal continuity was reestablished with a two layer gastrogastrostomy proximally and an end colostomy distally.

Postoperative Care. The animals required constant nursing care for the first twelve to twenty-four hours after surgery. Frequent endotracheal suctioning was necessary. Large quantities of blood and fluid were required continuously during this period. Although demonstrable blood loss was often less than 300 or 400 cc., no less than 2,000 cc. were given in any of the successful experiments in addition to 500 to 1,000 cc. saline solution. Infusion with levoarterenol was commonly employed during the first few postoperative hours. The administration of intravenous fluids was discontinued after the first two days, and the dogs were allowed to eat a diet consisting of sugar water and baby food. One million units of aqueous penicillin were given every twelve hours until death.

RESULTS

Period of Ischemia of the Homograft. In most cases, the period of devascularization was from sixty to seventy minutes. There were no survivors when ischemia exceeded ninety minutes.

Operative Mortality. Thirty-eight experiments were performed. Only five dogs lived longer than twenty-four hours after operation. All animals which did not die on the first day lived for five and a half days or longer. The remaining thirty-three died as an immediate consequence of surgery. In every early death, massive gastrointestinal hemorrhage was the primary cause of death.

Early Course After Homografting. The recipient animals generally remained in good condition during evisceration, placement of the graft and anastomosis of the vena cava. With restoration of the arterial supply to the graft, all dogs became profoundly hypotensive, usually with a blood pressure of 60 mm. Hg or lower, despite rapid transfusion. At least 1,000 cc. of whole blood was necessary to restore the blood pressure. Initially, the

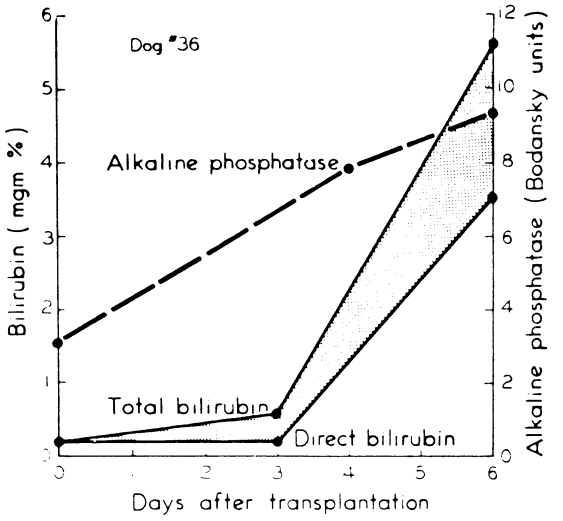


Fig. 4.— Development of chemical jaundice with rise in alkaline phosphatase, seen in three of the five long-surviving dogs.

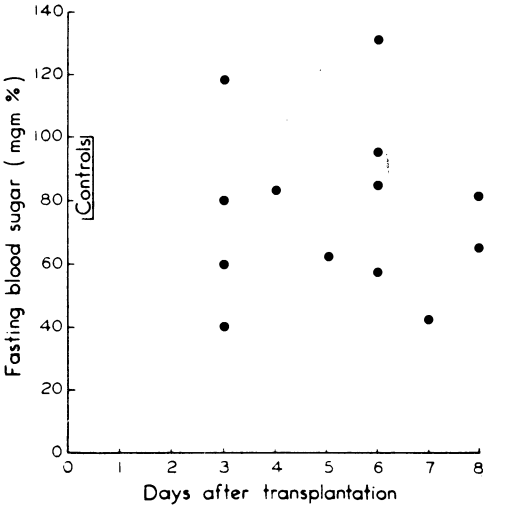


Fig. 6.— Fasting blood sugars in the five long-surviving dogs. Note usual absence of pronounced hypoglycemia.

color of the stomach and bowel was pink, but after thirty or forty minutes, faint cyanosis developed as blood sequestration occurred in the graft. Large quantities of clear or straw-colored fluid transuded the lumen of the gut. Lymphatics on the surface of the stomach and intestine became distended and formed serous blisters. After a time, the liver became dark in color and firmer than normal. Secondary bleeding from vascular anastomoses often developed. Slow infusion with levoarterenol was sometimes effective in combating the shock state Chlorpromazine, Arfonad,® reserpine and Pitressin® were of no value.

After closure, a profuse serous diarrhea ensued necessitating continuous blood and fluid therapy. In most animals, the diarrhea became bloody, and death followed from massive gastrointestinal hemorrhage in one to twenty-four hours. In the five surviving dogs, the diarrhea abated after twelve to eighteen hours. Further intensive care was not required.

In the early deaths, hemorrhagic gastroenteritis was invariably present at autopsy. All levels of the gastrointestinal tract were involved, but the most profound changes were usually in the duodenum. Histologic studies showed congestion, extravasation of blood, edema and sloughing of the alimentary tract. The other graft organs were also congested. Multiple punched-out duodenal ulcers were common.

The gastrointestinal hemorrhages were apparently not due to acute elevations of portal pressure. In five dogs, a portacaval shunt (Fig. 2) was placed between the donor portal vein and the recipient vena cava. Four of these animals died of alimentary bleeding.

Maximal Survival. The five dogs surviving the immediate postoperative period lived for five and a half, six, seven, nine and nine days, respectively. Fluid requirements abruptly decreased after the first day, and no parenteral therapy was given after forty-eight hours. High carbohydrate diet consisting of brown sugar solution and baby food was started on the third day. Vomiting was rare until just before death.

Physical activity of the dogs was somewhat reduced, but otherwise they seemed normal. Liquid colostomy drainage was continuous but not excessive in quantity. Terminally, in all dogs tarry or frankly bloody fecal drainage developed. The transit time of ingested material was rapid, and food was often undigested. Abdominal roentgenograms usually did not show an abnormal gas pattern. (Fig. 3.)

Most animals were febrile during their entire course. Pulse rates rose just before death. The dogs lost weight rapidly. After four to eight days, activity became sharply reduced, followed by vomiting and death in twelve to twenty-four hours.

Chemical Studies. In three of the five long-surviving dogs, jaundice developed on the fourth or fifth postoperative day. (Fig. 4.) Jaundice was progressive and involved parallel increases in both the direct and indirect reacting bilirubin. Corresponding rises occurred in the alkaline phosphatase. (Fig. 4.) In the other two animals which lived for nine days, the bilirubin remained within normal limits. (Fig. 5), although the alkaline phosphatase increased. (Fig. 5.)

Determinations of fasting blood sugars were performed at frequent intervals postoperatively. Hypoglycemia occurred uncommonly. (Fig. 6.)

Amylase values were abnormally high in three of five dogs. In all five animals, cholesterol levels rose, and in every case at least 30 mg. per cent esters were present at the time of the last determination before death. Protein and albumin: globulin ratios followed an unpredictable pattern. Blood urea nitrogen was elevated in only one animal. Calcium and phosphorus levels were within normal limits.

In all five dogs extreme electrolyte aberrations developed with severe hyperchloremic acidosis. Terminally, bicarbonate fell to as low as 7 mEq./L. and chlorides were as high as 120 or 130 mEq./L.

Urine Studies. Persistent albuminuria developed by the third day. The albumin loss was 4 plus at all times from the third day on. Concentrating power was retained, however, throughout the entire course, and shifting urinary pH's were demonstrated as late as the eighth day.

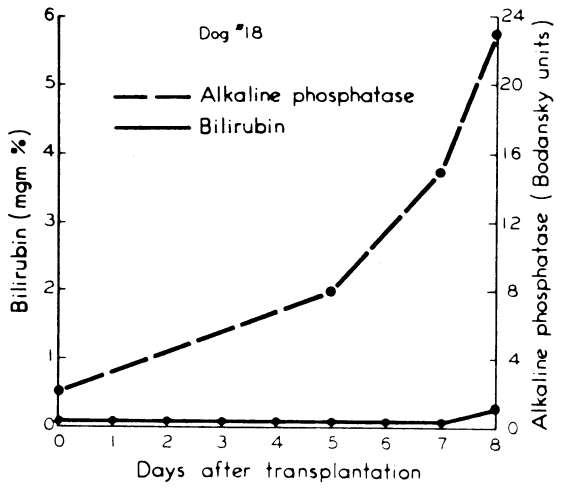


Fig. 5.— Absence of jaundice in two of the five long-surviving dogs. Note rise in alkaline phosphatase.

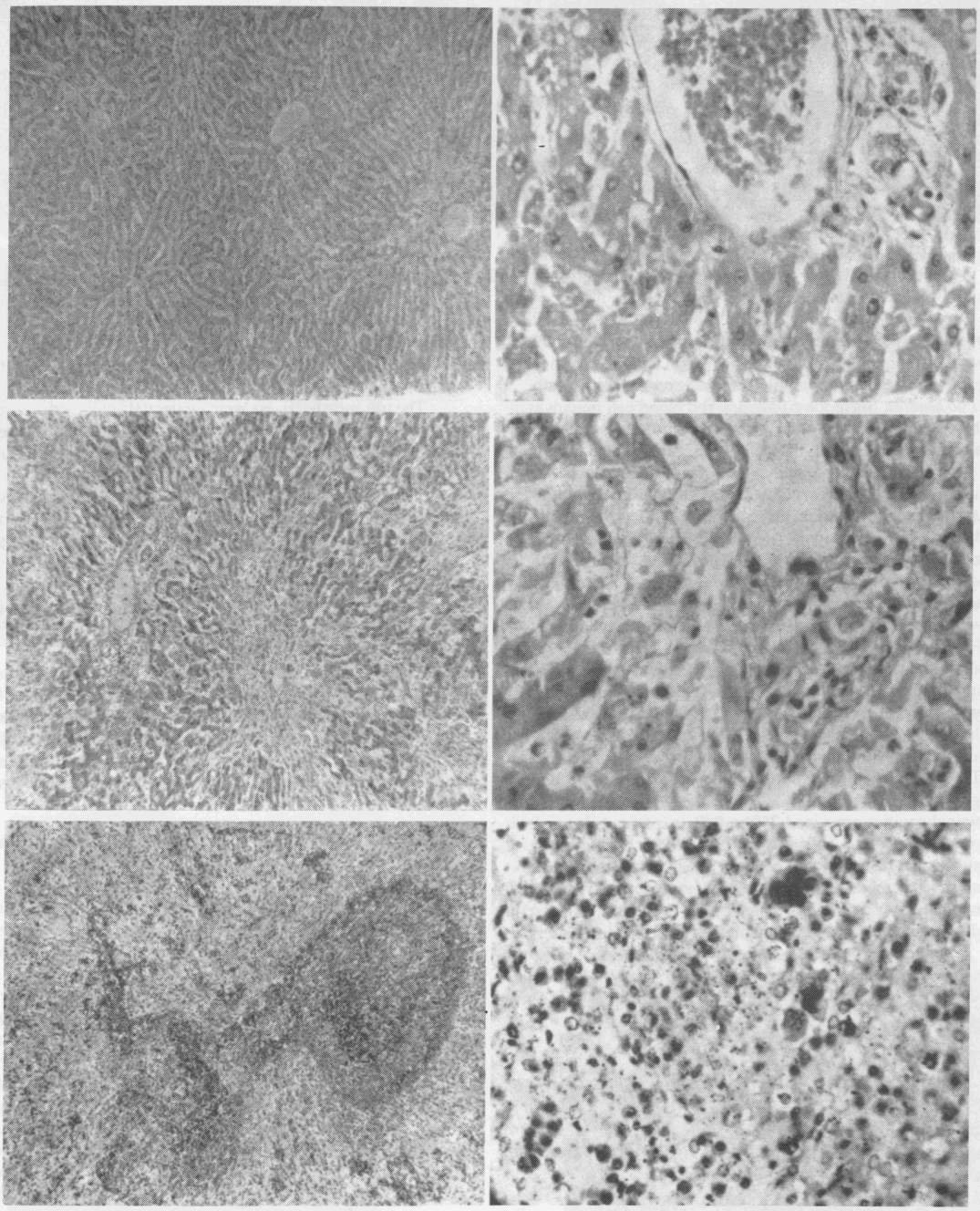
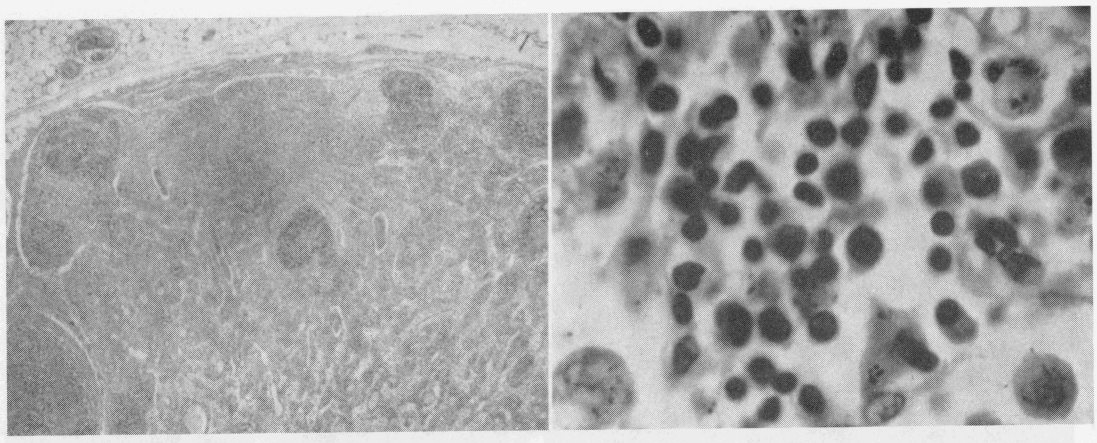


Fig. 7.— Liver after nine days, from Dog No. 18, in which jaundice did not develop. A, magnification X 65; B, magnification X 350.
Fig. 8.— Liver after seven days, from Dog No. 4, in which jaundice developed. A, magnification X 65; B, magnification X 350.
Fig. 9.— Donor spleen, after nine days, from Dog No. 19. A, magnification X 65; B, magnification X 350.



A B

Fig. 10.— Donor lymph node from mesentery of graft in Dog No. 26. Animal lived five and a half days. A, magnification X 30; B, magnification X 600.

In the three dogs with jaundice, urobilinogen disappeared from the urine by the fourth day, and bile appeared. In two dogs without jaundice, urinary bile was not detected, and urobilinogen was excreted until death.

Hematologic Studies. Since the circulating red blood cell mass was maintained by blood transfusions, the peripheral erythroid values could not be used as parameters of myeloid erythropoietic activity or blood loss. In the immediate postoperative period, each animal manifested a moderate leukocytosis due to a relative and absolute neutrophilia. Rather prompt subsidence of the leukocytosis was observed. In the animals which survived for nine days, the leukocyte counts had fallen on the sixth and seventh days to 4,500 and 4,700 per cu. mm., respectively. On the day before death, the white blood cell count was 4,200 per cu. mm. in one of these dogs. However, it had risen to 17,000 per cu. mm. in the other. Alterations in thrombocytes were not detected in these relatively short-term experiments.

Autopsy Findings. In the long-term survivals, postmortem examinations revealed abnormalities in both donor and recipient tissues. Two of the five dogs had small pleural effusions. Despite the fact that no intravenous fluid therapy was given after forthy-eight hours, each dog had severe generalized pulmonary edema. In two cases, there was also focal atelectasis. In all animals, the right ventricle had patchy granular areas, principally subepicardial. These lesions were previously shown to be due to the trauma of massive surgery [10].

The abdominal cavities contained 250 to 800 cc. of fluid. In some animals, this material was serous, and in others it was bile or blood stained. The gastrointestinal tract had profound changes. In all cases, there were mucosal or submucosal hemorrhagic areas at some level of the alimentary tract, usually associated with slough or edema. The changes were most marked in the duodenum and were accompanied by multiple deep ulcers in four dogs. In three dogs, the bowel was constricted; in the other two, it was dilated and appeared almost necrotic. In two animals, there was fat necrosis and other evidence of pancreatitis.

The transplanted livers were moderately enlarged and cut with a firm granular sensation. The spleens were slightly enlarged, somewhat mushy and generally dark. The kidneys and adrenals were normal in four dogs and congested in the fifth.

The gastrointestinal anastomoses healed well. There were no ruptures of the suture line. Venous anastomoses remained patent, but in two dogs there were partially occluding thrombi at the aortic anastomoses.

Mesenteric lymph nodes were greatly enlarged and edematous. In two animals, the mesenteric lymphatics were injected with methylene blue, but no lymphatico-venous communications could be demonstrated. The recipient lymph nodes, of which the mediastinal group were most extensively dissected, were not enlarged.

Histologic Findings in Homograft Tissues. The architecture of the liver was relatively well preserved. The two dogs which lived for nine days and did not become jaundiced had almost normal livers. There was slight central cell loss in some areas, and none at all in others. (Fig. 7.) The periportal mononuclear infiltrate, which was so prominent with homografts of the liver alone [11], was absent in some sections and present

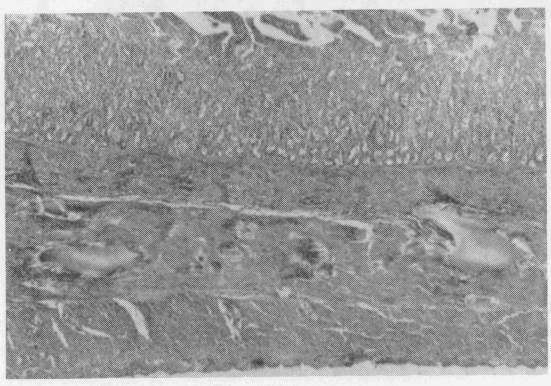


Fig. 11.— Small intestine of Dog No. 4, seven days after transplantation (magnification X 18). Note congestion, edema and superficial slough.

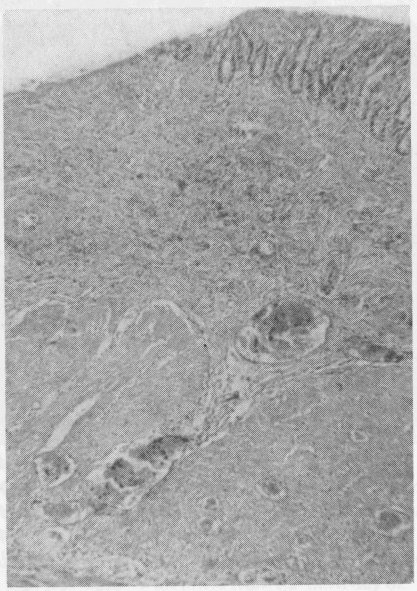


Fig. 12.— Duodenal ulcer in Dog No. 18, after nine days (magnification X 25).

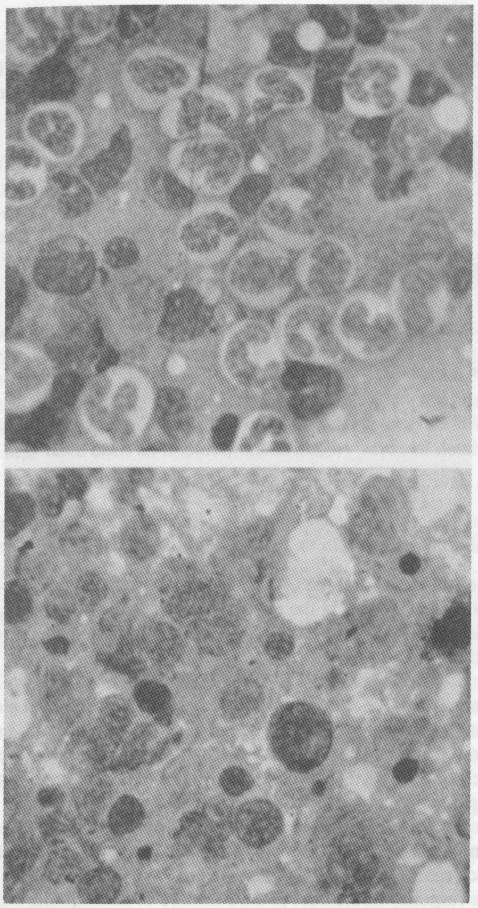


Fig. 13.— A, bone marrow from normal dog showing active granulopoiesis and erythropoiesis (magnification X 900). B, marrow from Dog No. 26, showing a cellular specimen with extensive replacement of normal myeloid elements by a relative and absolute increase in lymphocytes, reticulum cells and plasma cells (magnification X 900).

to a rather minimal degree in others. (Fig. 7.) Fatty metamorphosis which was thought to be due to nutritional depletion was present.

The three dogs which survived for five and a half, six and seven days and in which jaundice developed had more pronounced histologic abnormalities. There was a significant degree of central cell loss. (Fig. 8.) Periportal aggregates of mononuclear cells were more prominent. (Fig. 8.) However, even in this group, architectural distortion was far less than has been observed with transplantation of the liver alone.

Architecture of the spleen was preserved, although considerable congestion was present in four of the five animals. (Fig. 9.) Malpighian corpuscles were present (Fig. 9), but were sometimes compressed by congestion of the red pulp or partially replaced with a multitude of plasma cells. In the dogs which survived nine days, giant cells (Fig. 9B), which resembled megakaryocytes, and increased numbers of normoblasts were observed. In two dogs diffuse sheets of mononuclear plasmacytoid cells were seen in the red pulp.

The lymph nodes in the mesentery of the graft were studied. There was some distortion of the peripheral lymph channels because of the necessary ligation of the central drainage pathways near the coelic and mesenteric ganglia. Proteinaceous material and numerous macrophages filled the distended lymph vessels. The cortices were anatomically preserved with demonstrable follicles. (Fig. 10.) However, the follicles were reduced in size and number and infiltrated with a variable number of plasma and reticulum cells. (Fig. 10B.) The pulp also contained an increased number of plasma and reticulum cells. Throughout the nodes in

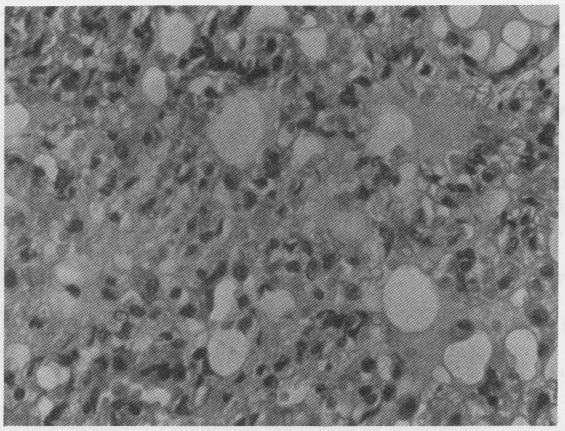


Fig. 14.— Lung from Dog No. 18, nine days after visceral transplantation (magnification X 350). Note pulmonary edema and proliferative thickening of alveolar septa.

two dogs, anomalous plasmacytoid cells with an abundant pink cytoplasm were present.

All portions of the gastrointestinal tract had similar changes, although these were most extensive in the duodenum. There was congestion of the entire wall of the viscus, usually with edema. (Fig. 11.) The mucosa was commonly ulcerated focally (Fig. 11), and in some cases there was massive slough. The duodenum in four dogs had deep punctate ulcers. (Fig. 12.) The wall was infiltrated with polymorphonuclear cells in the cases with slough, and there was always some degree of infiltration with mononuclear cells. In four of the five dogs, there was acute pancreatitis which was indistinguishable from that produced by a variety of experimental methods. In the fifth animal, there was pancreatic perivascular infiltration of plasma cells and evidence of fixed tissue proliferation.

Histologic Findings in Recipient Tissues. Marrow examinations revealed cellular specimens with striking abnormalities. (Fig. 13.) Normal myelopoiesis was largely replaced by relative and absolute increases in plasma cells, lymphocytes and reticulum cells. Plasma cells comprised approximately 15 to 20 per cent of all nucleated cells. In addition to the marked decrease in erythropoiesis and granulopoiesis, megakaryocytes although present were relatively infrequent.

Pulmonary edema was present in all animals. (Fig. 14.) Proliferation and thickening of the alveolar septa were present. In one animal, alveolar thickening was marked (Fig. 14), and multinucleated cells resembling megakaryocytes were seen. The kidneys showed no perivascular infiltration of mononuclear cells. However, there were occasional plasma cell aggregates in the perirenal and periadrenal tissues. There were focal myocardial infarcts with varying degrees of organization.

Recipient nodes from the mediastinum showed thinning of the cortex and a decreased number or total absence of follicles. (Fig. 15.) The nodes contained increased numbers of plasma and reticulum cells, often atypical. (Fig. 15B.) Skeletal muscle appeared normal.

COMMENTS

Previous studies in several laboratories have clarified the behavior of homografts of the individual organs which make up the complex graft used in the present study. The bowel [6], liver [8,11] and spleen [8] are usually rejected in five to ten days. The technical problems of transferring the multivisceral graft are less than with transplantation of the individual organs. Only three vascular anastomoses are involved, and these are of large caliber vessels. Despite this, the rate of success in obtaining dogs for long-term study was only five in thirty-eight, or 13 per cent.

In the failures, the usual cause of death was congestion and hemorrhage in the intestinal tract. The bowel congestion was apparently not due to acute portal hypertension, because the addition of portacaval shunts did not prevent its development. Similarly, the congestion could not be explained by excessive ischemia. Lillehei et al. [6] have shown that segments of the bowel can tolerate three or four times more ischemia than was inflicted under the conditions of these experiments.

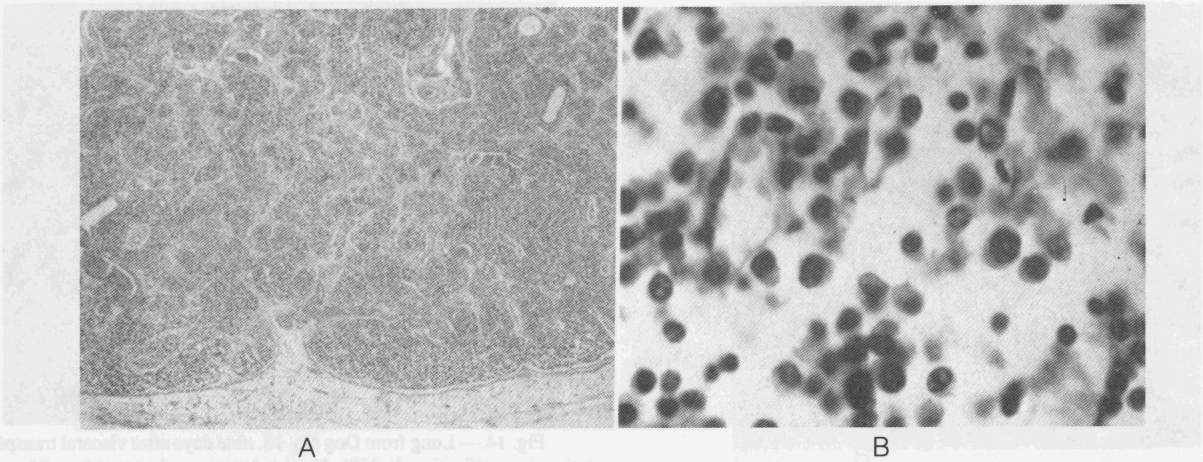


Fig. 15.— Recipient lymph node from mediastinum of Dog No. 36 after six days. A, magnification X 30; B, magnification X 600.

The failures may by explained by the denervation of the graft. The neuroanatomic state of the graft is depicted in Figure 16. All preganglionic fibers of the parasympathetic system were severed. The sympathetic ganglionic neurons were transported with the graft (Fig. 16) but were separated from all connections with the central nervous system. Popielski [9], Berger and Lium [1, 7] and Lillehei and Wangensteen [5] have studied the effects of sympathetic denervation of the bowel in dogs. After extirpation of the coeliac and mesenteric ganglia, mucous or bloody diarrhea commonly developed. The bowel became edematous and hyperemic with mucosal and submucosal petechial hemorrhages and slough. These changes are comparable to those seen in the present study. An additional contributory factor may have been the obstruction to outflow of lymph in the graft.

The behavior of the liver in the present studies is of interest when compared with the fate of single whole organ hepatic homografts as studied by Goodrich [3], Moore [8], Starzl [11] and their associates. In both types of experiments, most of the dogs which survived surgery lived for five to ten days. The events leading to death were not, however, entirely similar. When the liver was transplanted alone, bile production ceased after four or five days and, in our experience, jaundice invariably developed by the fifth or sixth day. In most cases, death was thought to be due to cessation of

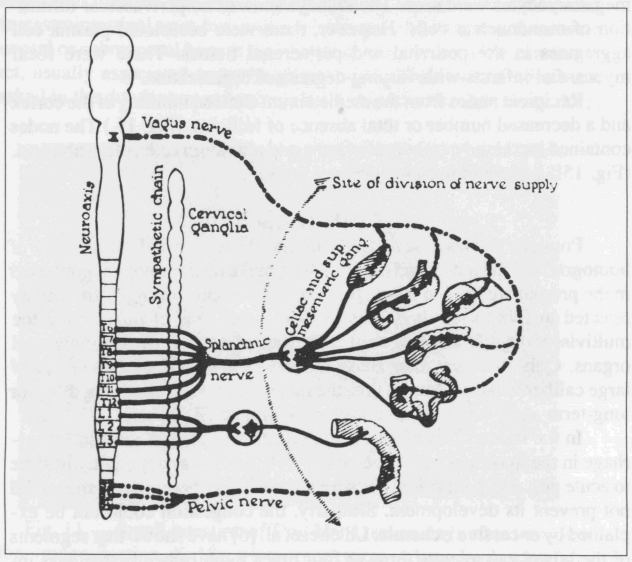


Fig. 16.— State of denervation of multiple organ graft.

function of the graft. In three of the multiorgan grafts, a similar pattern of early jaundice was seen. In the other two animals, however, jaundice did not develop during the nine days of survival. Urobilinogen persisted in the urine of these two dogs.

Some differences in the histologic appearance of the two kinds of liver homografts were also noted. The liver, when transplanted alone, was in our experience [11] commonly the site of extensive parenchymal destruction at the time of death. In every case, periportal aggregates of mononuclear cells were prominent. As part of the multiorgan homograft, hepatic parenchymal destruction was extensive in only one animal. Mononuclear cell aggregates in the periportal area were usually not prominent, and in the dogs in which jaundice did not develop, this histologic feature was virtually nonexistent.

The response of the host to the presence of the multiple organ graft also differed in some respects from the host reaction to the single organ liver homograft. After homografts of the liver, pulmonary edema occurred uncommonly [11]. Prompt and sustained leukocytosis developed. Despite cortical thinning and an increased number of plasma cells, the recipient lymph nodes retained a recognizable architecture [11]. In dogs with the multiple organ graft, pulmonary edema of such severity developed eventually that it may have been the direct cause of death in every case. The majority of these dogs ultimately had decreases in the white blood cell count. The increases in bone marrow plasma and reticulum cells were more striking, and there was apparent suppression of normal bone marrow activity. Finally, the architecture of the recipient lymph nodes was altered markedly. A definite cortex could rarely be identified, and the follicles were entirely absent in some dogs.

The difficulty of evaluating the inter-reaction between immunologically competent host and graft tissues has been emphasized by Billingham [2]. Histologic changes in donor or recipient organs could represent activity by either host or recipient tissues or both. Despite this limitation in the interpretation of data, there is evidence that the relation to the host of the multiple organ graft is quantitatively different than that of the single organ liver graft. The greater degree of structural and functional preservation of the liver in the multiple organ graft suggests mitigation of the rejection process.

Conversely, evidence for a graft versus host rejection response is stronger in the recipients of multiple organs than in those receiving the liver alone. In animals receiving the liver alone, there was no evidence of functional deterioration of any of the host organ systems. After multiple organ grafts, there was evidence of host organ failure. Examples included suppression of bone marrow activity and the invariable development of pulmonary edema. However, the precise roles of graft and host tissues in the production of these changes cannot be ascertained from our data. Evaluation of the extent of host versus graft and graft versus host reactions will depend on studies in which either the host or the graft is rendered immunologically incompetent by radiation or other means.

SUMMARY

It was technically possible to perform simultaneous homotransplantation of multiple visceral organs including the liver, spleen, pancreas, omentum and the entire gastrointestinal tract. Arterialization of the cooled graft was accomplished through the donor aorta which was removed with the graft and attached to that of the recipient dog. Gastrointestinal hemorrhage after surgery accounted for a high operative mortality and was thought to be due to denervation of the graft.

The five dogs which survived the immediate trauma of surgery lived for five and a half to nine days. After the second day, these animals were physically active and able to resume oral alimentation. In three dogs, there was metabolic evidence of rejection of the liver. In two others, jaundice did not develop.

These observations were compared with chemical, hematologic and pathologic data obtained in previous experiments involving homotransplantation of the liver alone. In some cases, there was less evidence of host versus graft rejection after the multiple organ transplants. Other data in the present study suggested the possibility that a significant graft versus host reaction may have been an important contributory cause of death.

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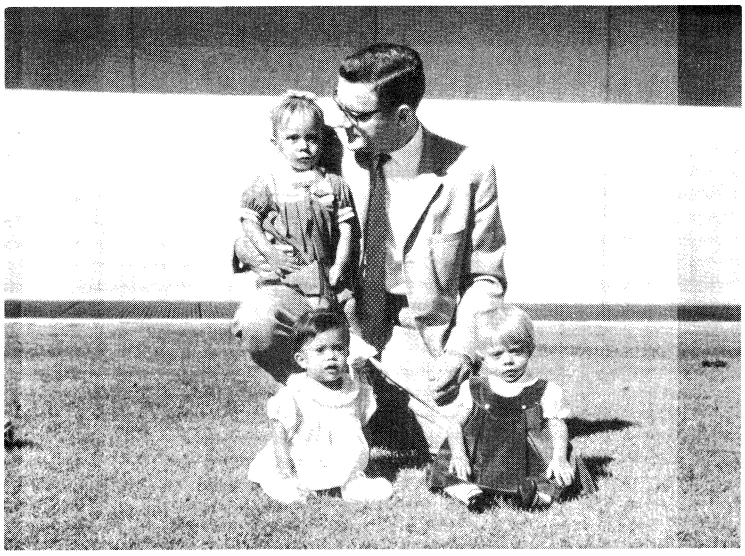
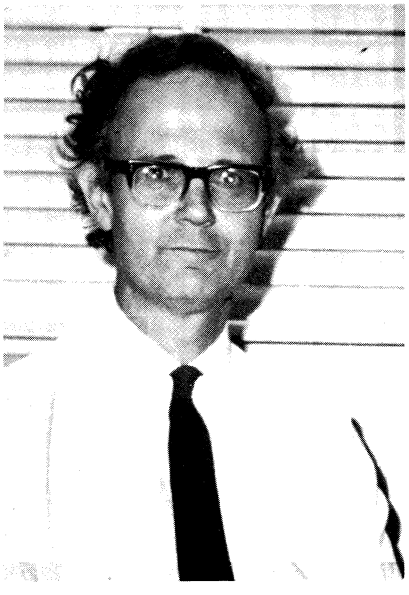


In the late autumn of 1967, Dr. Carl Groth, then a fellow in transplantation at the University of Colorado, was photographed with three of the first human survivors after liver replacement. The child on Dr. Groth's knee lived for 400 days before dying of metastases from the hepatoma for which she was originally treated.

In unmodified dogs, the changes in hepatic blood flow caused by rejection were measured with a xenon washout technique. Flow invariably was reduced with rejection. The flow alterations were correlated by Professor K.A. Porter of St. Mary's Hospital and Medical School, London with light microscopic and ultrastructural changes detected in serial biopsies. A more coherent and dynamic view of rejection emerged from these studies. Porter still is at St. Mary's where he is Professor of Pathology.



Kendrick A. Porter is Professor of Pathology, The St. Mary's Hospital and Medical School, London. In September 1963, Ken Porter and TES (then at the University of Colorado) met at a meeting about renal transplantation held at the National Science Foundation in Washington, D.C. The results being obtained with renal transplantation in Colorado were so striking that Dr. Porter came to Denver to review what was going on there. From that visit came a collaboration that has lasted for more the two decades. Dr. Porter's chapters in Starzl's books on renal (1964) and hepatic (1969) transplantation were monographs in their own right. Dr. Porter's talents soon turned to an assessment in various experimental models of the effect upon liver morphology of portal venous as opposed to systemic blood. The histopathologic changes became the most important end points in many of the complex experiments that were used to examine the hepatotrophic hypothesis (see Part IV). As befits an Englishman, Dr. Porter's principal side interest is gardening.

Studies of blood flow and ultrastructural changes in rejecting and nonrejecting canine orthotopic liver homografts

Surgery, 63: 658-68, 1968

Carl G. Groth, K. A. Porter, Jean B. Otte, Pierre M. Daloze, Thomas M. Marchioro, Lawrence Brettschnider, Thomas E. Starzl

It has long been thought, on the basis of observations with vital microscopy, that a sudden decrease occurs in the blood flow of skin homografts at the time of their rejection.[15,22] There is evidence that the same is true in homotransplanted whole organs. In 1953, Dempster[2] demonstrated a striking loss of small branches of the arterial tree in renal homografts which were excised during their rejection and studied by means of angiography. More than 10 years later, Kountz and associates[7] and Williams[26] and their associates obtained serial flow measures in canine kidney homografts with a radioactive-hippuran technique. In untreated recipients, there was a decline in total renal flow which was most dramatic at the time of rejection.

Shortly after, it was reported that rejection after clinical renal homotransplantation was accompanied by changes which could be readily explained only by ischemia. These included a drop in urine sodium concentration, an increase in urine urea and creatinine concentration, oliguria, a reduction in creatinine clearance, and arterial hypertension.[21] The findings, which simulate those which can be produced experimentally by partial occlusion of a renal artery, were in patients who had developed rejection while receiving azathioprine therapy. They were quickly reversed with the addition of prednisone. Subsequent studies in dogs have confirmed both that a reduction in blood flow is coincident with renal homograft rejection[5,6,14,16,24] and that this change can be prevented or reversed with appropriate immunosuppressive therapy.[6,16]

Such studies have raised the possibility that ischemia is an important general mechanism of rejection. In the present study this question has been examined in liver transplants by determining hepatic blood flow in both treated and untreated recipients of orthotopic homografts. In addition, a separate electron microscopic study was made with serial liver biopsies from untreated recipients, with the special objective of looking for ultrastructural abnormalities in either large or small blood vessels which could explain hemodynamic changes.

Methods

Experimental groups. Mongrel dogs, with an average weight of 8 to 16 kilograms, were immunized against hepatitis and distemper and used as homograft recipients. Orthotopic hepatic transplants were performed, as previously described,[20] with pentobarbital anesthesia combined wih the tranquilizer, phencyclidine hydrochloride. Dogs that died of technical complications or intussusception were excluded. Serum bilirubin, alkaline phosphatase, serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and complete blood counts were obtained frequently in all animals. The patency of vascular anastomoses was established at autopsy.

Group 1. The liver flow was studied in 10 unmodified recipients. In 8 of these, serial postoperative measurements were done daily or every other day until the death of the animal; in the other 2, measurements were done only on the first posttransplant day. In 9 of these experiments the liver blood flow was also measured in the donor animal on the day before transplantation.

Group 2. Five recipients were administered antilymphocyte globulin (ALG) and azathioprine. ALG was given daily for 5 days pretransplant and 30 days after operation; subsequent injections were twice a week. The preparation and the dosage of ALG was the same as in previous reports from this institution.[4,19] Azathioprine was given daily from the day of transplantation. The dose varied between 1 and 8 mg. per kilogram of body weight per day, depending on the white blood cell count of the animal. Blood flow measurements were done for as long as 19 days, usually every third day.

Group 3. Five untreated recipients were used for pathologic studies. The donor liver was biopsied before transplantation. Post-operatively, biopsies were obtained every second or third day until death. Each tissue sample was divided into 3 pieces. The first piece was immediately diced up into tiny fragments, fixed in osmium tetroxide, processed, and embedded in Araldite. Sections 0.5μ thick were cut, stained with Azur II, and examined by light microscopy. Later, very thin sections were examined in a Siemens Elmiskop 1A electron microscope. The second piece was snap-frozen at -70°C., and sections cut on a cryostat were examined in ultraviolet light after treatment with fluorescein isothiocyanate-conjugated antisera to canine IgG and complement. The third piece was fixed in 10 percent formalin, processed, and embedded in paraffin wax. Sections were exam-

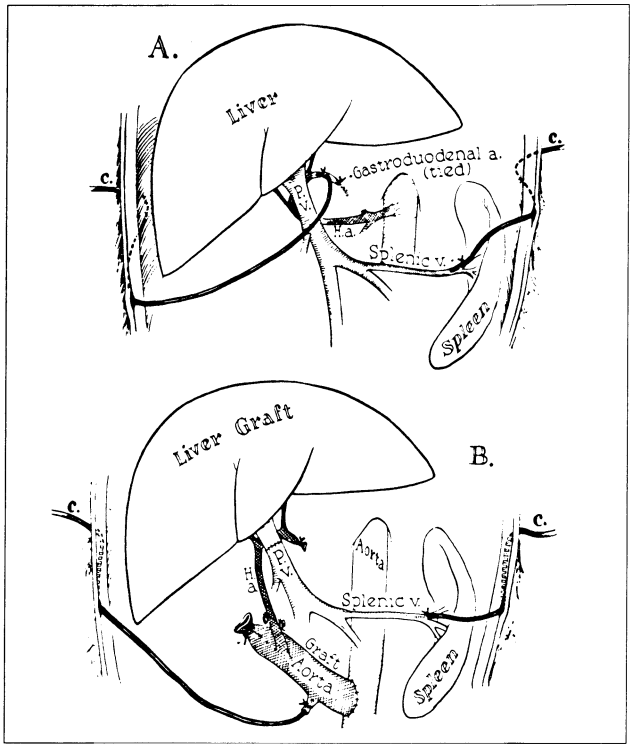


Fig. 1.— The placement of indwelling catheters in the portal and hepatic arterial systems in the donor (A) and the recipient animal (B). Note that the gastroduodenal artery in the donor is tied to ensure delivery of the isotope solely to the liver.

ined by ordinary light microscopy after they had been stained with hematoxylin and eosin, van Gieson's method for elastic and methyl green pyronin.

Flow studies. The liver blood flow was studied in the unanesthetized state by measuring the washout of the inert, radioactive gas, xenon-133. The procedure was essentially the same as that used by Hollenberg and Dougherty.[3] Approximately 500μc of Xe^{133} dissolved in 1 to 3 ml. of isotonic saline was rapidly injected through indwelling catheters in the portal and the hepatic arterial systems (Fig. 1), giving a peak radioactivity over the liver of 30,000 to 100,000 counts per minute. To obtain return to background radioactivity between the injections, 10 to 30 minutes were required. The washout of the isotope was monitored externally with a collimated, 1 inch sodium iodide crystal scintillation detector mounted above the liver approximately 1 cm. from the dog. A linear rate meter and a recorder were used.

To obtain the disappearance rate constant, K, the curves were replotted in a semilogarithmic system where $K = \frac{\log 2}{T_{1/2}}$,

$T_{1/2}$ being the half time of disappearance. In most instances the first 9/10 of the semilogarithmic plot was an apparently straight line, only the last part being curved (Fig. 2, A). In some others, the plot was a curved line throughout (Fig. 2, B). The first type was regarded as an essentially one-compartment system, and $T_{1/2}$ was assessed directly from the straight part of the plot. In the other type a second compartment was subtracted graphically, and $T_{1/2}$ for this and the resulting first compartment were assessed. The flow in the second compartment was always less than 20 percent of that in the first, and in the results only the flow values for the first compartment are given. Knowing K, the blood flow was calculated as follows[23]:

$$\text{Flow (ml. per 100 Gm liver per minute)}^* = \frac{L \times 100}{P} \times K$$

Where L is the partition coefficient between tissue and blood (this was calculated for the prevailing hematocrit according to Veall and Mallett[25] with the relative solubility values of Xe^{133} in plasma, erythrocyte, and liver given by Conn.[1]), and P is the specific gravity which for the liver is 1.02.[13]

If the solubility of Xe^{133} in liver tissue were to change during rejection, a systematic error would be introduced in the flow calculations. The relative solubility of the isotope in normal and rejected liver tissue was, therefore, compared in two experiments, in vitro. After the livers had been perfused with lactated Ringer's solution until they became clear of blood, equal aliquots of homogenized tissue were mixed with equal amounts of Xe^{133} in gas sampling tubes at 37° C. for 30 minutes. The average ratio between the activity in homogenates of normal liver and rejected liver homografts were 0.91 and 1.02, respectively. It was concluded that the same solubility value could be used throughout without inducing an error exceeding 10 percent.

Concomitant with the blood flow studies, cardiac output was measured by an indicator dilution method with Xe^{133} as the tracer substance.[17] An isotonic saline solution of the isotope was infused at a known constant rate (R) into the systemic venous circulation, and samples were taken in the

* It should be emphasized that flow values obtained on portal and arterial injection (designed FPI and FAI, respectively) do not represent an absolute measure of fractional flow from either source, as will be discussed later.

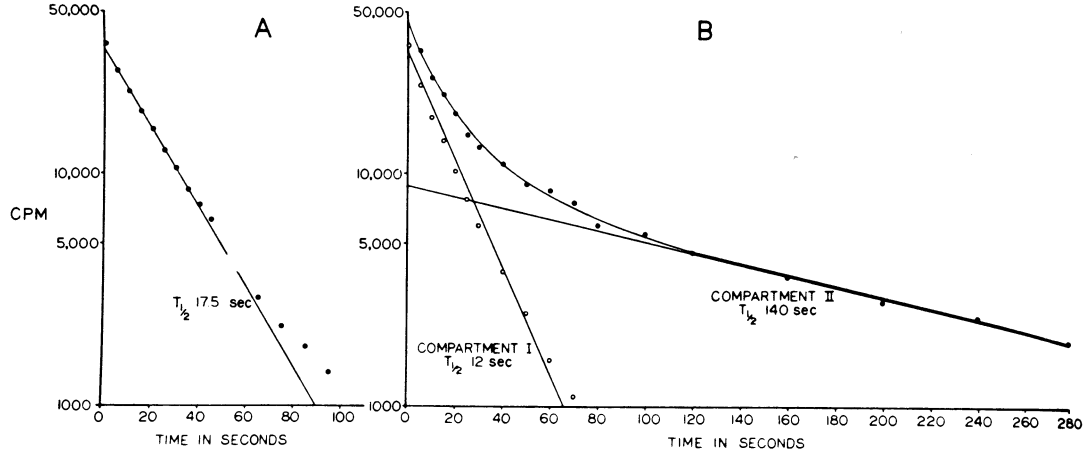


Fig. 2.— Semilogarithmic plots of typical disappearance curves following injection of Xe133. Usually most of the plot was a straight line (A). In some instances, however, it was a curve that could be resolved into 2 compartments (B).

pulmonary artery through a cardiac catheter. After counting the activity per milliliter in the infused solution (I) and in the mixed venous blood (B), the cardiac output was calculated according to the formula:

$$\text{Cardiac output milliliters per minute} = \frac{I}{B} \times R$$

Results

Liver blood flow in normal dogs. The mean FPI and FAI in 19 normal dogs studied with the present technique were 221± 52 (SD) milliliters per 100 Gm. tissue per minute and 178 ± 50 (SD) ml., respectively.

Liver blood flow in untreated recipients. In the 9 experiments in which studies were obtained before and one day after transplantation, the hepatic blood flow was usually slightly lower in the recipient than it had previously been in the donor. The declines in FPI and FAI were of the same magnitude. Part of the difference in flow could be accounted for by lower cardiac outputs in the recipient animals. Mean values are given in Fig. 3.

Of the 8 dogs that were studied serially after transplantation, 7 died of rejection after 6 to 10 days, with a mean survival of 8 days. In these animals, there was a progressive deterioration in liver function. Postmortem microscopic examination of the livers showed typical features of rejection.[20] A decrease in liver blood flow occurred concomitantly with the deterioration in hepatic function (Fig. 4). The decreases in FPI and FAI were ultimately 41 and 54 percent, respectively. The mean changes in the cardiac output were small (Fig. 4) and could not account for the changes in flow.

One of the untreated recipients lived for 22 days and ultimately died with pneumonia and wasting. Until death the dog's liver chemistries were normal, except for an elevated alkaline phosphatase. There was no histologic evidence of rejection. During the first 3 postoperative days, this animal had a subnormal liver blood flow as well as a reduced cardiac output. Subsequently, both values became supernormal for a few days and then settled within normal limits until the last day of study.

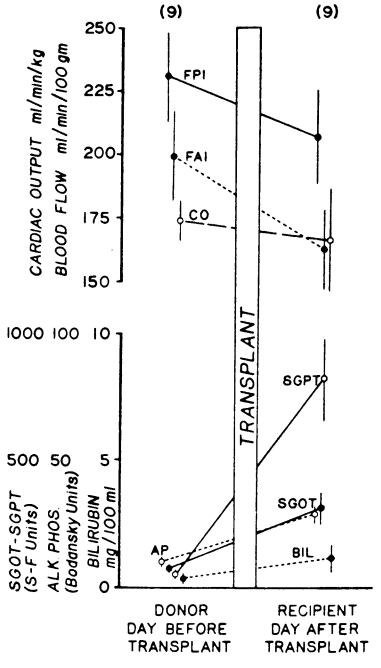


Fig. 3.— Liver blood flow as measured by portal injection (FPI) and hepatic arterial injection (FAI) in the donor and recipient on the days before and after transplantation. Cardiac output and liver function are also shown. Mean values ± SE in 9 experiments are depicted.

Liver blood flow in recipients given immunosuppression. One of the 5 dogs treated with ALG and azathioprine died with typical features of rejection 13 days postoperatively. None of the remaining animals had a clinically diagnosed rejection during the first 20 days. One died after 14 days of an anaphylactic reaction during a blood transfusion. One died after 4 months and 4 days due to an intestinal volvulus; histologically, the homograft was normal except for a few mononuclear cells in the portal tracts. The other 2 are still alive after 7 months.

The changes in blood flow in the dog that died of early rejection were similar during the first 9 days to those in unmodified recipients; the flow decreased concomitantly with a deterioration in liver function. However, on the eleventh posttransplantation day, the flow improved at the same time as the liver chemistries had begun to return toward normal. The cardiac output was unchanged during these events. On the thirteenth postoperative day, the day of death, there was again a drop in blood flow along with a deterioration in liver function. At this time, cardiac output was also

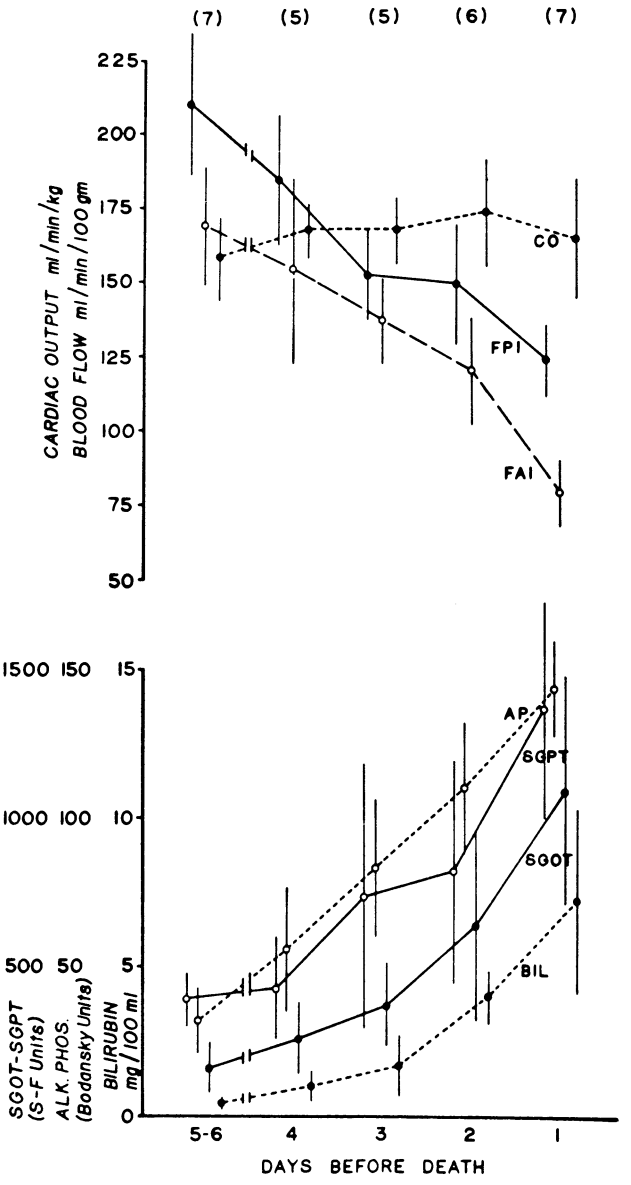


Fig. 4.— Liver blood flow, cardiac output, and liver function in 7 unmodified recipients that died of rejection. The mean values ± SE are shown, as well as the number of observations (in parentheses) for each day.

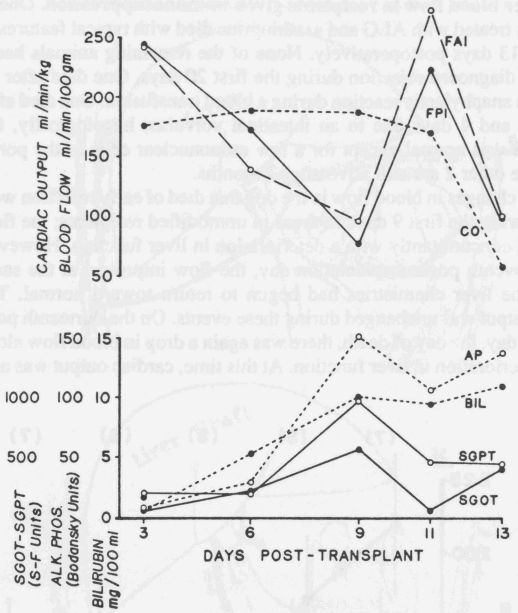


Fig. 5.— Liver blood flow, cardiac output, and liver function in a recipient receiving immunosuppression. The animal died of rejection 13 days after transplant. Note the concomitant improvement of flow and liver function on Day 11, indicating reversibility of the flow changes.

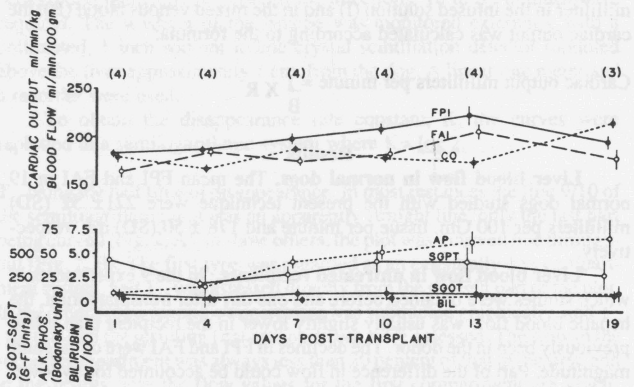


Fig. 6.— Liver blood flow, cardiac output, and liver function in 4 recipients which received immunosuppression and had little or no evidence of rejection. Mean values ± SE are given, as well as the numbers of observation (in parentheses).

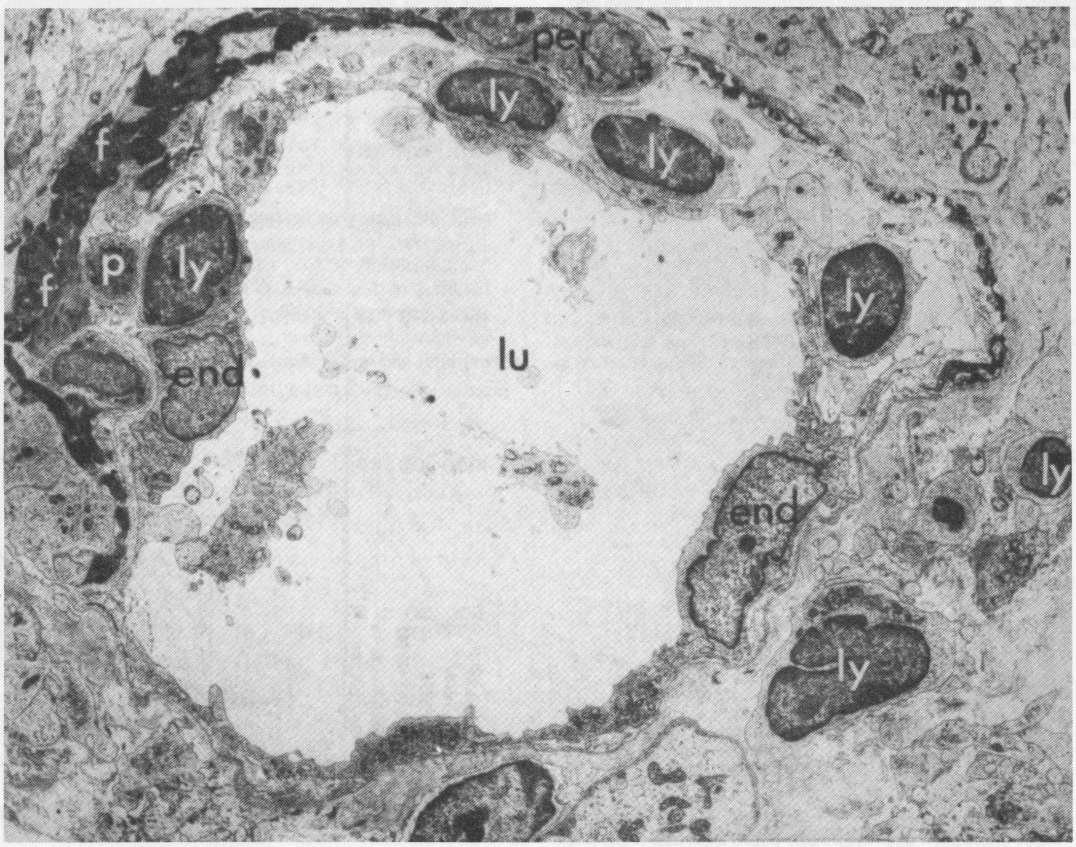


Fig. 7.— Biopsy, 4 days after transplantation, of an hepatic homograft in an untreated dog. Electron micrograph showing a central hepatic vein. Lymphocytes (*ly*), platelets (*p*), and fibrin (*f*) lie beneath the endothelial lining (*end*) of the vessel. Fluid and cells lie in the perivascular space (*per* = pericyte; *m* = macrophage; *lu* = lumen of vein). (Lead stain. x 2,250.)

markedly decreased (Fig. 5).

The remaining 4 animals, which had little or no evidence of rejection as judged by liver function tests, did not have any significant changes in blood flow or cardiac output during the study period of 13 to 19 days (Fig. 6).

Serial pathologic changes in rejecting liver homografts. The 5 untreated dogs had evidence of a typical rejection, as judged by liver chemistries. All died in 4 to 9 days. The biopsies obtained on Day 2 showed slight dilatation and congestion of the centrilobular sinusoids and damage to a variable number of the hepatocytes in the central zones of the lobules. In the injured cells there was dilatation and destruction of the rough endoplasmic reticulum, clumping of swollen mitochondria, and shedding of cytoplasm. Macrophages containing ingested fragments of cytoplasm and organelles were present in the tissue spaces. These cells, together with neutrophil polymorphonuclear leukocytes and occasional lymphocytes, were common around the portal and central veins. The lymphocytes were ordinary, small lymphocytes, with few organelles in their cytoplasm. No immunoglobulins were detected at this stage.

By the fourth day cellular infiltration was marked around the portal and central veins. Large lymphoid cells with pyroninophilic cytoplasm that was full of polyribosomes, but lacking in rough endoplasmic reticulum, were common and could be seen beneath the endothelium of the veins (Figs. 7 and 9) and in the space of Disse. Some were touching the endothelial cells. The tenuous walls of many of the centrilobular sinusoids appeared disrupted (Fig. 8), and fibrin lay in the venous subendothelial spaces (Figs. 7 and 9). A few plasma cells with abundant rough endoplasmic reticulum were present, particularly in the portal tracts. The centrizonal hepatocytes were now necrotic, and in one hepatic homograft the centrilobular bile canaliculi lacked microvilli and contained bile plugs. Immunofluorescence showed no deposits of immunoglobulin G and complement in the vessel walls, but the cytoplasm of several of the infiltrating cells "stained" positively for IgG.

The later biopsies were less instructive. Necrosis of hepatocytes was widespread, and large numbers of lymphoid cells, many macrophages, some polymorphs, and plasma cells were present in the portal and central zones and lying between the necrotic hepatocytes. The lumina of several of the central veins were completely blocked by collections of infiltrating mononuclear cells. Cholestasis was pronounced in the better-preserved peripheral areas of the lobules. In one hepatic homograft, 8 days after transplantation there was deposition of IgG and complement in the walls of several small arteries, and, ultrastructurally, a homogeneous, finely granular deposit was present between the endothelium and the internal elastic lamina. By light microscopy a few of these affected arteries showed "fibrinoid necrosis" of their walls. No deposits of immunoglobulin or complement were found in the other homografts.

Discussion

Techniques for liver blood flow determination during rejection must be independent of liver function. The measurement of the clearance of inert radioactive gases fulfills this requirement and has the further advantage of permitting daily studies with a minimum of manipulation of the animal. The method does not differentiate clearly between the fractional contribution to total liver blood from the hepatic arterial and portal venous sources since there is variable presinusoidal or intrasinusoidal communication of the 2 vascular systems.[12] Consequently, the washout of the isotope is not due solely to tissue perfusion by the vascular system which receives the

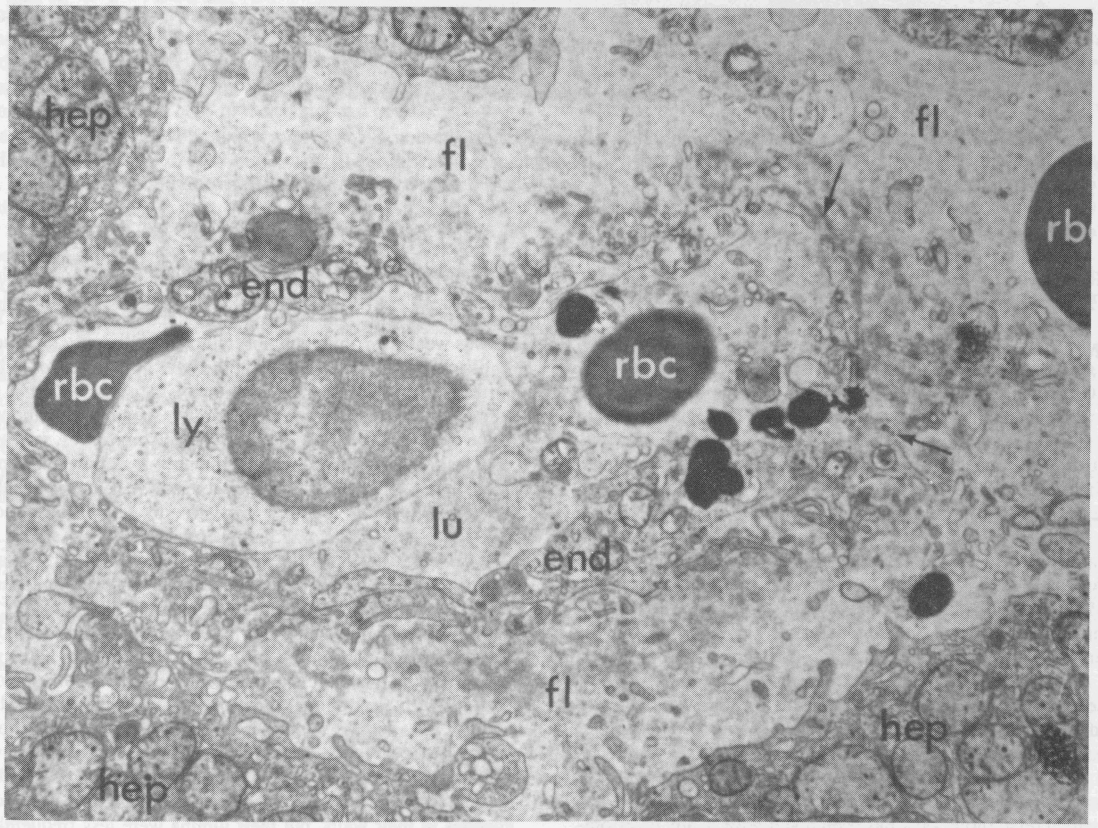


Fig. 8.— Biopsy of canine hepatic homograft, 4 days after transplantation. Electron micrograph showing a centrilobular sinusoid. The endothelial lining (*end*) is ruptured at the point marked with arrow. The space of Disse is wider than normal and contains a red cell (*rbc*) and fluid (*fl*). A lymphoid cell (*ly*) and two erythrocytes (*rbc*) are present in the lumen (*lu*) of the sinusoid. The adjacent hepatocytes (*hep*) are injured, as shown by swelling and clumping of their mitochondria and loss of their rough endoplasmic reticulum. (Lead stain. x 6,000.)

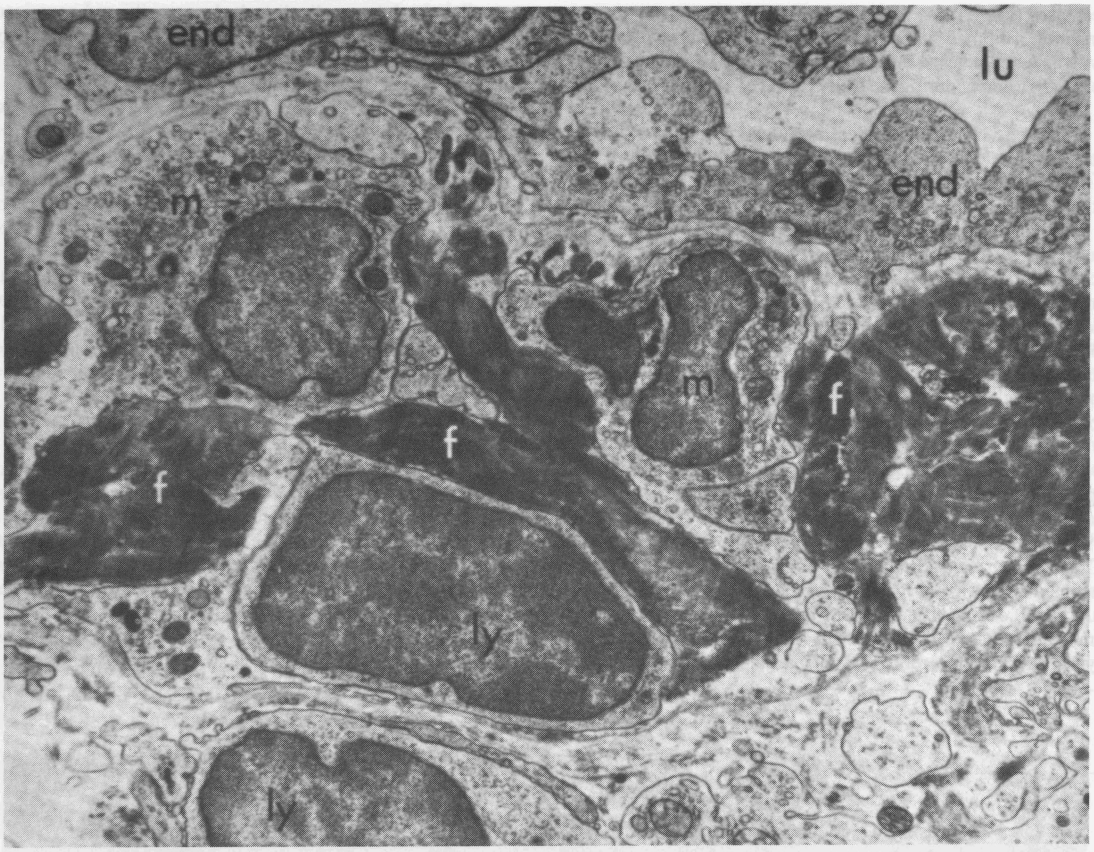


Fig. 9.— Biopsy of untreated canine hepatic homograft, 4 days after transplantation. Electron micrograph showing part of wall of a vein in a small portal tract. The wall is infiltrated by lymphocytes (*ly*), macrophages (*m*), and fibrin (*f*) (*lu* = lumen of vein; *end* = endothelial lining cells.) (Lead stain. x 6,000.)

injection. Nevertheless, the fact that the flow values actually differ with the route of isotope administration indicates that the intrahepatic mixing is incomplete.[3,13]

The results obtained with this method after homotransplantation of the liver show that total liver blood flow decreases significantly at the time of rejection. Conversely, the decline was not seen in one untreated animal which did not have a rejection, and it was prevented altogether in other dogs which received adequate immunosuppression. On one occasion, flow fell during rejection in a treated animal and was later restored when liver chemistries began to improve.

Some indirect evaluation of the importance of relative changes in portal and hepatic arterial circulation can be deduced from observations of Hollenberg and Dougherty.[3] They found that total occlusion of the hepatic artery decreases both the FPI and FHI to about 2/3, while occlusion of the portal vein decreases FHI to approximately 1/3 of control values. The recorded changes in our animals would thus seem to be best explained by a decrease in both portal and arterial flow. The decrease might well, however, be more marked in one of these systems than in the other.

In those animals which had clinically evident rejection, the reduction in liver blood flow did not precede the characteristic abnormalities of liver function. The temporal relation between flow alterations and the biochemical changes was almost absolute. Thus, the order of events was not completely comparable to that reported in rejecting canine kidney homografts in which renal blood flow has been noted prior to any deterioration in function.[6,14]

Nevertheless, the findings confirm earlier suspicions that there is an important component of ischemia in the rejection of liver homografts. On the grounds of histologic findings, it was initially suggested that blood flow was choked off at a sinusoidal level.[8,18] Subsequently, Moore and his associates[9] proposed, on the basis on angiographic evidence, that lesions of larger vessels within the portal tracts were responsible for devascularization of discrete, rather large areas of hepatic parenchyma. The hypothesis was weakened, although not refuted, by the rarity of structural abnormalities of these larger vessels in the liver homografts of either treated or untreated recipients.[20] In the latter study, the possibility was raised again, on the basis of preliminary electronmicroscopic observations, that injury to the sinusoidal bed was responsible. In some areas, mononuclear cells were found to be fused to, and presumably damaging, the centrisinusoidal endothelium. The possible analogy was pointed out between these findings and the peritubular capillary lesions described by Kountz[7] and Porter[11] and their associates in rejecting renal homografts.

The fact that blood flow is rapidly restored upon reversal of rejection could be explained by an initial mechanism of heightened vasomotor reactivity, although an attempt by Moore and his associates[9] to affect the course of rejection by intra-arterial administration of a vasodilating agent was not successful. Consequently, the principal effort in the present study was to find an anatomic explanation for the hemodynamic changes. The findings with electronmicroscopy suggest that the reduced blood flow is mainly due to damage to the veins and sinusoidal bed of the homograft. The sinusoids appear to have actually disrupted, while the central veins became narrowed by masses of cells in their lumina and by cells and fibrin lifting the endothelium from the vessel wall. The portal veins showed similar changes in their walls, but their lumina were less frequently filled with infiltrating cells. Platelet aggregates were not seen in these vessels.

These findings are compatible with the hypothesis that acute rejection in the untreated hepatic homograft is predominantly cell mediated. We have no evidence that circulating antibody plays a significant role at this time. Deposition of IgG and complement on and in vessel walls was not

apparent until 8 days after transplantation, at a time when the rejection process was far advanced. Paronetto and associates,[10] in their studies on auxiliary hepatic homografts without a portal blood supply, also found that gammaglobulin did not appear in the walls of the hepatic arteries until the second week after transplantation. There is still no proof that the lymphoid cells damage the vascular endothelium of the hepatic graft, but it is probable that this occurs. No evidence was obtained in these orthotopic liver grafts of the periportal "piecemeal" necrosis described by Paronetto and co-workers.[10]

Summary

The blood flow in orthotopic canine liver homografts was investigated in awake animals with a Xe^{133} washout technique. Cardiac output was also studied. Ten dogs which received no immunosuppressive treatment lived for as long as 22 days (mean 8.5 days). With the onset of rejection, as diagnosed by elevations in bilirubin, SGOT, and SGPT, there was a significant decrease in both components of liver blood flow which could not be accounted for by changes in cardiac output.

These findings were correlated with the immunofluorescent and ultrastructural findings of five additional experiments in which biopsies of homografts were obtained before and every second day for 4 to 8 days after orthotopic liver homotransplantation to untreated recipients. It was found that lymphoid cell infiltration of the grafts commenced at about 4 days after transplantation and resulted in damage to the walls of the portal and central veins and to the centrilobular sinusoids. Localization of immunoglobulin G and complement in the homograft was a rare and late phenomenon.

Another 5 recipients were given immunosuppression with horse antilymphocyte globulin and azathioprine. When there was no biochemical indication of rejection, the liver blood flow was essentially unchanged in animals studied for as long as 19 days after transplant. The findings in these 3 experimental groups indicate that decreased blood flow and consequent ischemia is an important factor in the rejection of liver homografts, that such changes can be prevented by effective immunosuppression, and that an anatomic basis for the flow alterations could be the disruption of the centrilobular sinusoidal walls and the intraluminal and subendothelial accumulation of host lymphoid cells in the central and portal veins.

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This picture of Henri Garnier was taken at Garnier's winter home in April 1978, at the time of the Franco-American Surgical Conference in Marakesh, Morocco. Six weeks previously, Garnier had undergone a cholecystectomy in Paris, at which time the liver was thought normal. However, ten days after this picture was taken, symptomatic hepatic metastases from a bronchogenic carcinoma were discovered. Garnier died during the following Christmas season.

The French surgeon, Henri Garnier, dreamed of clinically applying orthotopic liver transplantation. In his 1965 publication,* he described the technique of liver transplantation in pigs. In the follow-up paper by Cordier, Garnier and others, Garnier documented the long survival (>35 days) of an animal without immunosuppression. The work was known to the English workers in Bristol and Cambridge. Garnier died during the Christmas season, 1978, with widespread metastases from a bronchogenic carcinoma.

*Garnier H et al: *CR. Acad Sci* (Paris), *260:* 5621-23, 1965

La greffe de foie orthotopique chez le porc. Premiers résultats
Orthotopic grafting of the liver in pigs. First results

Mem. Acad. Chir. (Paris), *92:* 799, 1966

Gaston Cordier, Henri Garnier, Jean-Paul Clot, P. Camplez, J.-P. Gorin, Ph. Clot, J.-P. Rassinier, M. Nizza and Roger Lévy

Translated by John Novier

For almost two years we have been trying to perfect the orthotopic grafting of the liver in pigs.

This animal was chosen because of the relative similarity of the hepatic anatomy and biliary ducts with that of man.

In the first experimental series dealing with 23 animals, we studied the various technical problems relating, above all, to various venous shunts and vascular and biliary-enteric anastomoses. These examination of these problems in 23 autografts *performed under normothermia* have yielded the following results:

— 18 peri-operative deaths

— 4 survivors of 2, 3, 9 and 26 days, respectively

— 1 long term survival

This series was the subject of a communication to the Academy of Sciences on 24 May, 1965, communication made by our mourned mentor, Dean Gaston Cordier.

Today, we will discuss the results obtained in 20 *orthotopic grafts without any immunosuppression.*

We have used pigs of the Large-White species that weigh an average of 60 kgs.

1. *Anesthesia.*— Half an hour before the operation, the two animals are premedicated with an injection of 25 mg of alimemazine (théralène), 100 mg of atarax, and 1/4 of a mg of atropine.

At first the animal was given fluothane for a few minutes through a special mask. As soon as it lost consciousness, it was placed on its back. A vein of the ear was catheterized enabling us to inject 0.50 g of penthotal and 15 mg of D-tubocurarine. After oxygenation, the animal was intubated, (sometimes not without difficulty) and placed on a R.P.R. type respirator (frequency 24 per minute and average tidal volume of 550 cm^3). A polyethylene gastric catheter was inserted.

In several pigs we placed an indwelling urinary catheter. The procedure was very difficult, but it enabled us to monitor urinary output during and after the operation which was always excellent: 1,500 cm^3 during the operation and 2,000 during the first twenty-four hours. The urine never was hemorrhagic.

Anesthesia is maintained by repeated, small injections of penthotal, and by perfusion at 2 per 100 of viadril in glucose solution. Because of the irregularity of electrical conduction of the pig heart, cardiac function is stabilized by pronestyl.

Moreover, at the outset of the operation the animal receives 2.5 mg of heparin per kilo.

2. *Resuscitation and perioperative monitoring.*— Glucose solution,10 per 100, and blood are perfused through a venous catheter. This catheter provides, among other things, a venous access for reversal of anesthesia. We use porcine blood taken the day before. The problem of blood group incompatibility is not as prominent as in man. Although the pig has several blood groups (more than 50), the cross-matching techniques have demonstrated the frequency of agglutination. Yet, a more extensive study of this problem is presently in progress. The E.K.G. and blood pressure are monitored.

— Blood pressure is monitored by means of a catheter placed in the femoral artery and connected to an electronic manometer.

— Blood loss is replaced as carefully as possible. Two phases of the operation are monitored with particular care:

a) Recipient hepatectomy because the liver weighs approximately 1,500 g and removal of this organ results in a loss of approximately 800 cm^3 of blood.

b) Removal of clamps and "hooking-up" of the transplanted liver. When the anastomoses are complete, we perfuse 12 g of hemocaprol and then 8 g again at the very end of the operation.

3. *Upon waking up*, the pig is placed under an electric blanket. As a matter of fact, the temperature of the animal is then low, approximately 32° C (normal temperature 38° C), and it seems to us that the pig tolerates hypothermia very poorly. In order to avert a decrease in temperature, as soon as the liver is connected we transfuse the recipient through a site other than the liver with blood warmed in a double boiler to 30° C.

The pig is watched continuously during the first 24 hours. Blood pressure is maintained by infusions of glucose solution,10 per 100 and 30

per 100, to which we add insulin (10 to 15 I.U.) and by blood transfusions.

During the first night, we also infuse 250 cm^3 of THAM and 500 cm^3 of mannitol. When the outcome is favorable, the animal eats again spontaneously at the end of 24 hours. For about eight days, he will receive 10 million units of penicillin. The thoracic drain is removed at 24 hours.

4. *The operative protocol* , the source of many disappointments, has been modified several times. We report here the protocol,used presently, that has been proposed by Starzl and his associates. The procedures are performed by two surgical teams so that the donor liver can be transplanted immediately following the recipient hepatectomy.

A) *Regarding the donor:* With the animal placed on its back, we make a right thoracoabdominal incision.

The various vessels are dissected. This presents no problem for the suprahepatic vena cava which has a very long intrathoracic segment. On the other hand, the subhepatic vein often is very difficult because it is accompanied by a strip of hepatic parenchyma which extends inferiorly.

At the level of the hepatic hilum, it is common to find rather large lymph nodes that should be removed. Yet, dissection of the hilum is not particularly difficult except in cases of anomalous arteries or bile ducts as with duplicate right hepatic arteries and right hepatic ducts.

Vessels are dissected 5-6 cm, and dissection of the hepatic artery is extended to the level of or beyond the origin of the gastro-duodenal artery.

After freeing the vessels and the suspensory ligaments of the liver, a large cuff of diaphragm is prepared where the suprahepatic vena cava passes through it. To do so, it is necessary to tie several diaphragmatic vessels.

Once the graft is freed, both common iliac veins and one common iliac artery are cannulated for the purpose of extracorporeal cooling. This extracorporeal circulation is begun approximately twenty minutes before the recipient hepatectomy.

B) *Regarding the recipient:* The same approach is used, but, first, we isolate the two jugular veins which, in swine, are very large as well as the left femoral vein and the femoral artery on the same side in which we have inserted a catheter for measuring arterial pressure.

The dissection of the various hepatopetal and hepatofugal vessels is fairly similar to that performed on the donor. Also, we dissect the hepatic artery which is always of sufficient size to permit anastomosis.

In particular, we must exercise care regarding hemostasis since the animal is heparinized and also with regard to lymphatic stasis since there are many lymphatics the loss of fluid from which can be considerable.

Before proceeding to the recipient hepatectomy, several shunts must be established.

a) A femoro-jugular shunt between the left femoral vein and the left jugular vein in order to decompress the vena caval system below the clamped subhepatic vena cava.

b) A spleno-jugular shunt between the splenic vein and the right jugular vein. We still perform a splenectomy. The purpose of the shunt is to decompress the portal system when this vessel is clamped. Once the hepatectomy has been performed, the donor liver, cooled and weighed, is placed in the recipient. In order to place the graft into the circulation, anastomoses are performed in the following order:

— suprahepatic vena cava: the anastomosis is made with two continuous everting sutures of 4-0 silk,
— subhepatic vena cava: same technique,
— portal vein: same technique,
— then, the hepatic artery; this last anastomosis is made with interrupted sutures of 7-0 silk.

For a time, we altered the sequence of anastomoses as follows:

— suprahepatic vena cava,
— portal vein,
— subhepatic vena cava,
— hepatic artery.

This sequence, however, regularly resulted in a few minutes in ventricular fibrillation and death.

Actually, the average time of hepatic ischemia does not exceed 30 min, on the average. The cuff of the diaphragm is then sutured with interrupted sutures of 1-0 flax. Biliary drainage is created either by cholecystoduodenostomy or by choledochoduodenostomy.

As soon as the liver is functioning again, the two shunts are clamped and the cannulae are removed.

The wound is closed one hour after the transplanted liver has been functional and after a specimen of the liver has been taken in order to determine the state of the hepatic parenchyma at this stage.

The thorax and abdomen are drained.

The total operative time is approximately four hours and thirty minutes.

C) *Graft preservation during the ischemic period remains to be solved.* We have resolved this problem by decreasing the temperature of the liver by extracorporeal circulation with an oxygenated thermal exchanger.

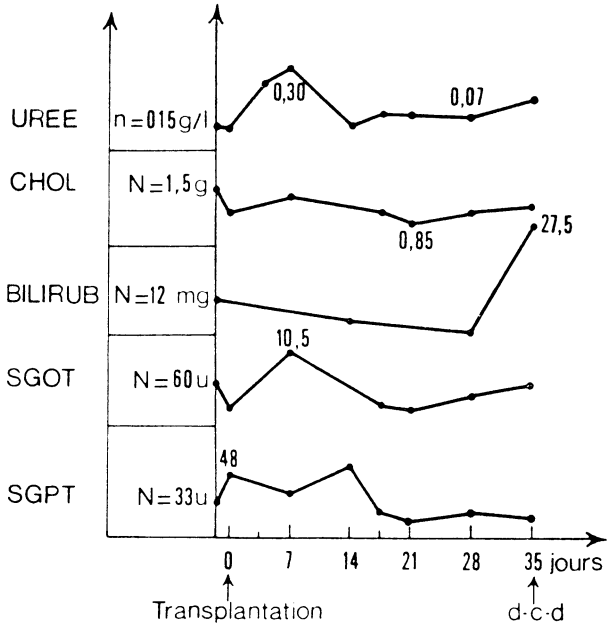

Fig. 1.— Curves of urea, cholesterol, bilirubin, transaminases.

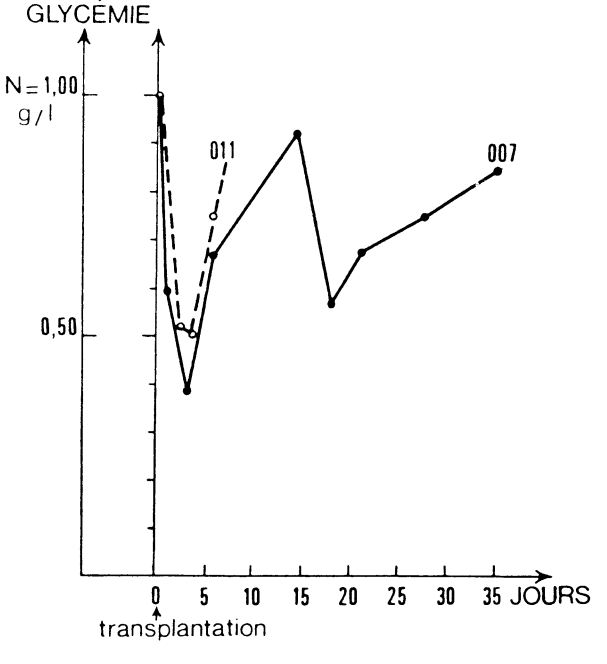

Fig. 2.— Study of glycemia in animals 007 and 0011.

We utilize the following circuit:

— The pump is filled via two venous catheters introduced into the common iliacs after separation of the return circulation of the lower extremities.

Once the blood has been arterialized, it is cooled and returned by perfusion into one of the common iliac arteries. The catheter is passed high enough into the aorta to prevent too much fluid from leaking out. Also, as extracorporeal circulation is initiated, we clamp the supradiaphragmatic aorta and the suprahepatic vena cava.

Perfusion is monitored by determinations of:

— frequency of pulsation and, by means of this, the circulatory flow,

— temperature of the cooling bath,

— rotational speed of the oxygenator disks,

— time of perfusion,

— liver temperature measured by means of a thermocouple connected to a galvanometer by a quartz thread; the probes are introduced into the right and left lobes.

It should be noted that in order to prime the pump we use 3 liters of isotonic sodium chloride, and that losses of fluid within the intracorporeal circuit are corrected by additions by equal quantities of the same fluid. Thus, the blood is considerably diluted.

The following conditions are standard:

— Outflow: 70 pulsations/minute, or 2.15 liters

— Bath temperature: mean, 7° C

— Approximative outflow of oxygen: 11 liters/mn

— The time of perfusion is determined by surgical requirements. The mean is 30 minutes, but in fact 15 minutes are sufficient to bring the liver to the desired temperature (see graphs).

Generally, the temperature is between 18 and 20° C. It is useless to cool the graft more than this considering the relatively short duration of ischemia. Moreover, when the liver is very cold it can effect cardiac function.

Utilizing this technique, we are able to transplant a liver that is perfectly pink throughout. We intend to continue to use this procedure.

Results

Our 20 attempts of orthotopic transplantation have yielded the following results:

— 7 perioperative deaths,

— 7 deaths within the first twenty-four hours,

— 3 deaths after thirty-six hours,

— 1 death after forty-eight hours,

— 2 survivors of thirty-five and twenty-one days respectively.

1. We have an explanation for the 7 perioperative deaths.

A. In three cases death occurred as a result of ventricular fibrillation. This fibrillation has always appeared at the same moment, i.e., when we place the liver in the circulation after having completed the anastomoses of the suprahepatic vena cava and the portal vein. Removal of the clamps from those two vessels causes, within a few minutes, a slowing down of the heart and then irreversible fibrillation in spite of the use of a defibrillator. This type of accident never has occurred again when we first anastomosed the suprahepatic vena cava and then the subhepatic vena cava before anastomosing the portal trunk. It is possible that, after removal of the clamps from the portal trunk, a considerable volume of refrigerated blood passes from the hepatic parenchyma to the heart and causes fibrillation.

When we first reestablish continuity of the vena cava system, the liver becomes warmer progressively; owing to this fact, at the time of the removal of the clamps from the portal trunk, the hepatic parenchyma has reached a higher temperature. Furthermore, the blood which has gone through the liver is still warm, because it is mixed with blood which came from the inferior vena cava system. The impact on the heart then is small or altogether absent.

B. One death was caused by a total volvulus of the small intestine during intraoperative manipulation.

In fact, we take the measure of placing all the intestinal ansae in a "small intestine bag" in order not to be hindered by them. In this instance, this resulted in an irreversible volvulus of the small intestine.

At the present time, we are satisfied to protect the small intestine in cloths soaked with tepid saline.

C. Three deaths are attributable to portal hypertension, and this constitutes one of the most difficult technical problems to be resolved. In fact, as is the case with dogs, pigs do not tolerate portal hypertension.

We have tried to resolve this problem by placing a shunt between the splenic vein and the right jugular vein, but this procedure is not always satisfactory, probably because the flow in the shunt is insufficient. Little by little, the small intestine assumes a venous coloration and becomes totally inert.

Thanks to supportive techniques, we are able to maintain a normal arterial pressure in the animal, but after a certain time it drops progressively. Shock ensues, even when we had time to perform the anastomoses and, consequently, decompress the portal system.

In pigs, unlike dogs, it is anatomically feasible to establish a temporary portacaval shunt.

For this reason, we are considering the placement of a shunt originating directly in the portal trunk or the temporary clamping of the superior mesenteric artery.

2. This portal hypertenstion very likely is the cause:

A. Among the three deaths that occurred during the first 24 hours, shock developed progressively and eventually led to death. No significant hemorrhage occurred in these instances although in each the bowel became purplish red.

B. In three other cases, postoperative death was related to hemorrhage. As a matter of fact, autopsy demonstrated an copious volume of blood in the peritoneal cavity, 2 liters or more. In each case, we found a small dehiscence of the continuous everting suture on the posterior sub-

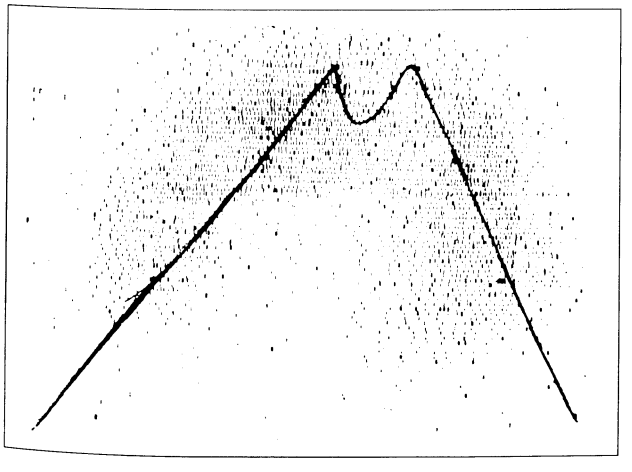

Fig. 3.— Hepatic scintigraph of the control animal.

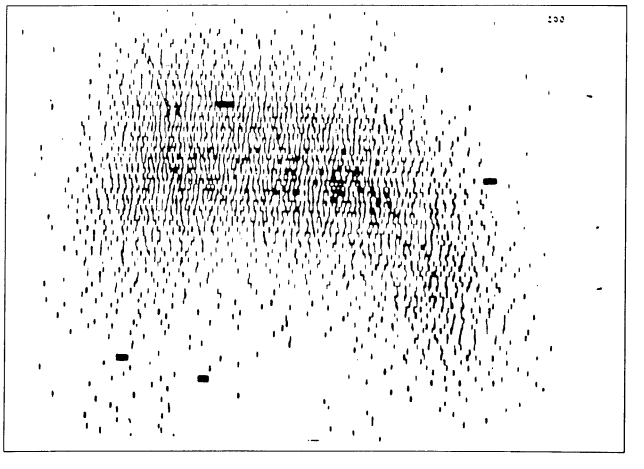

Fig. 4.— Hepatic scintigraph made on the twelfth day on subject 007.

hepatic vena cava. This anastomosis is the most difficult to perform because it is practically impossible to isolate perfectly the two venous segments inasmuch as in certain instances an actual sheath of hepatic parenchyma extends inferiorly as far as the region of the renal veins.

Nevertheless, after unclamping, the anastomoses always are sealed perfectly. It is possible that thrombolytic phenomena are involved in the onset of this hemorrhage.

Up to now, it has not been possible to examine the coagulation aspects of this procedure, but shortly we intend to address this problem.

C. Finally, one death within the first twenty-four hours is related to an error in postoperative care, as the animal was not monitored adequately.

3. It is more difficult to explain the three deaths that occurred between the twenty-fourth and thirty-sixth hours.

It is possible that in one of these cases bronchial congestion developed and that in another case a fulminant hemorrhage took place as the animal struggled when blood was taken.

We have no explanation for the third death. We lost an animal at forty-eight hours as a result of an accident. The pig died of suffocation.

4. The two survivors of thirty-five and twenty-one days have been studied on the biologically: urea, cholesterol, bilirubin, transaminases.

A. The blood urea, normally approximately 0.15 g per liter, never was markedly elevated, 0.30 in one case, 0.75 in another.

Cholesterol, normally 1.50 g, on the other hand, tended to remain relatively low.

Bilirubin, normally 10-12 mg per liter, remained at the upper limit of the normal range until late in the postoperative period.

In the animal who lived thirty-five days, the levels increased from the twenty-eighth day to reach 27.5 mg on the day of death.

With regard to the transaminases, in one case they remained normal, or sub-normal: 35 units; in the other case they were elevated moderately: 70 units (Fig. 1).

We also studied the level of sugar, normally 1 g per 1000. At first, the levels were extremely low: 0.40 or 0.50 per 100 and reached more normal levels numbers, 0.80 g, around the twenty-fifth day (Fig. 2).

B. In one of our animals on the tenth day we performed hepatic scintigraphy (see plates). Thanks to the courtesy of both Professor C. Kellerson and Professor A. Desgrez the picture that we obtained was close to that of the control animal (Fig. 3 & 4). The kupfferian clearance measurements in the transplanted animal demonstrated extremely rapid purification, perhaps faster that in the control animal. At autopsy, we noted that in both cases anastomoses were perfectly patent, the liver was moderatly decreased in volume and of pale coloration; at autopsy the weight was 300-400 g less than the weight at the time of transplantation.

C. Histological study of the parenchyma demonstrated the following (Dr. C. Calmettes):

"The cellular architecture is disrupted by edema. Cells appear to be very retracted, sometimes irreversibly altered. These changes are diffuse. All the vessels contained in the fragments are dilated considerably. In some places, there are small foci of old hemorrhagic necrosis. Other samples, kidneys, lungs, heart, are normal."

Two facts should be noted:

a) Autopsy of the animal who lived thirty-five days revealed a well-defined approximately 1 cm diameter perforation of the greater curvature of the stomach. Such a perforation also has been noted by Starzl and perhaps is related to certain ulcers observed after portacaval anastomosis. It is possible that the transplanted liver loses its power to detoxify certain metabolites, but this remains to be proven.

b) The two long term survivors seem to have been unusually long considering the unexpected appearance of phenomenum of rejection. At first, we thought that the animals were "brothers", but a careful investigation ruled out this hypothesis.

Consequently, at present we have no satisfactory explanation for this prolonged survival, except for the possible role of splenectomy in the attenuation of immunologic responses. A more detailed study for this purpose will be undertaken.

Conclusions

The authors report their experience, unique to their knowledge, regarding the orthotopic transplantation of the liver in pigs.

For the time being, this experience confirms the conclusions of other groups that have examined this problem in dogs.

Surely, the present results on pigs are poor, but this is due, in great part, to the fact there exist no or very little data concerning experimentation in this animal; each step of this research has raised as many original problems.

On the other hand, since technical problems have been overcome after many disappointments, it would appear that the great anatomical and physiological similarity between the pig and human livers justifies this study.

• • •

We express our gratitude to Doctors F. Le Goazio, E. Besins, as well as to Miss A. Aurousseau, M.-J. Perrot, G. Supernant and I. Lagoutte for their very valuable collaboration.

(*Study No. 8 of the Department of Surgery, C.H.U. Pitié-Salpetrière Hospital* [Professor Agrégé, M. Mercadier], *work performed in the laboratory of applied radiology of Jouy-en-Josas* [Director: M. M. Nizza], *Department of Sanitary Protection of the C.E.A.* [Director: Dr. H. Jammet], *Institut d'Immunologie et de Carcinogenetique de Villjuif* [Director: Professor G. Mathé]).

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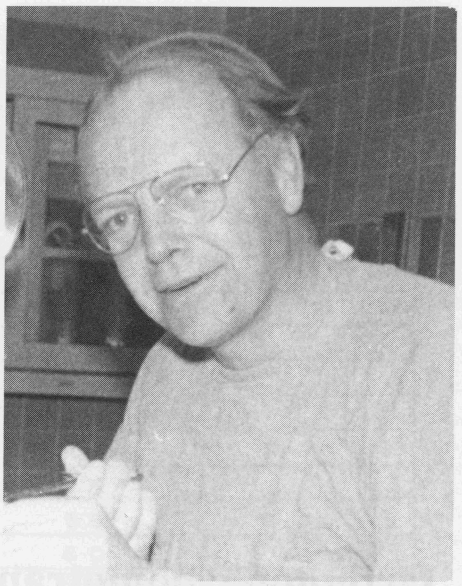
J. H. Peacock



This picture of John Terblanche was taken in the surgical research laboratories of the University of Colorado during his sabbatical leave in Denver in 1977.

J. H. Peacock was a senior lecturer at the University of Bristol in England. With him was a young South African surgeon, John Terblanche, now Professor and Chairman of the Department of Surgery in Capetown. Peacock and Terblanche were aware of Garnier's work and quickly reproduced and extended it. Their pig experiments provoked some of the most interesting discussions at a Colston International Congress on Liver Disease held in Bristol in 1967.

Peacock is retired and lives in Ubley, near Bristol, England.

Orthotopic homotransplantation of the liver in the pig

In: Read, A. E. (ed.): *The Liver*. London, Butterworth & Co., Ltd., 1967, pp. 333-6

J. H. Peacock and J. Terblanche

The purpose of this paper, which is the first of three to deal with the experiences of a team in Bristol working over the last 15 months on experimental liver transplantation in the pig, is to introduce the reasons for our choice of this animal, to detail some of the difficulties we have encountered and to give the results in a relatively small series of forty-one animals in which this procedure has been attempted.

Although we have had fairly extensive experience of clinical liver surgery, we were new to the field of transplantation in general and the liver in particular, and as a result our aims were essentially unambitious and twofold only.

1. To establish the technical experience and ancillary services necessary for us to undertake liver transplantation with a reasonable degree of success.

2. To study the process of rejection in the pig's liver.

Our excuses for presenting somewhat prematurely the limited amount of data that we have available in the form of three papers stem partly from the belief that some of the difficulties we have encountered are peculiar to the pig and as such may be of value to other workers who may contemplate using this animal but mainly from the fact that Professor K. Porter, who was to have read a paper this afternoon cannot unfortunately be here, and we have as a result persuaded two other members of our group Drs. A. C. Hunt and M. O. Symes to extend somewhat the contributions they would otherwise have made.

The pig as an experimental model

The pig was chosen as the experimental animal for a number of reasons. It is omnivorous, easily obtainable, cheap to maintain and like man has no hepatic vein sphincters.[1] Moreover, preliminary anatomical and biochemical investigations indicated not only that the jugular, iliac and splenic veins were suitable for by-pass systems, but also that the blood biochemistry with minor exceptions was essentially comparable to that of man. Further factors which influenced us were that fresh blood was available daily in quantity at two local abattoirs, and that a University School of Veterinary Science existed which was prepared not only to help us in maintaining a continuous supply of animals but also to act as a holding centre should we be fortunate in achieving long term survivors.

The animals used are outbred and equivalent to a random sample of humans. They have been obtained from two distinct sources. The recipients are bred at the Veterinary Farm and are a cross between a Large White boar and a Wessex sow. The donors are bought from a number of breeders through local markets and are crosses between either a Large White or a Landrace boar and crossed Large White/Landrace sows. The weight of the animals used initially was in the region of 20-30 kg, but during the later stage of the programme it was found easier from the point of view of cannulation to use animals of a somewhat larger size and 35-40 kg animals are now regarded as being most suitable.

Technique

The transplant is undertaken by two teams working simultaneously on donor and recipient animals. Anaesthesia is induced with halothane through a nose cone and maintained after intubation on closed circuit gas, oxygen and minimal halothane. Vena caval and portal by-pass systems are inserted between the iliac and splenic veins in the abdomen and the two jugular veins in the neck. The portal by-pass is more critical in the pig than in the dog, as occlusion of the portal vein for four minutes can lead to circulatory collapse in some animals. The arterial blood pressure, central venous pressure and E.C.G. are monitored continuously and half hourly estimations of blood pH, O_2 saturation, PCO_2 and glucose are made during and after operation. The donor liver is prepared by isolation of its major vessels, the hepatic artery being left attached to a 5 in. segment of thoracic aorta. The liver temperature is lowered to between 6°-12° C by perfusion through the portal vein of 8 litres of ice cold Ringer lactate at 1 litre/minute immediately the arterial supply to the liver is disconnected. Both donor and recipient animals are heparinized using a dose of 1.5 mg/kg/body weight and only fresh heparinized blood is used for transfusion. All the anastomoses are by standard techniques and biliary drainage is by a cholecystoduodenostomy. Prior to closure a splenectomy is performed. Hepatic out-flow obstruction has not been seen except in one animal in which there was an increase in systemic venous pressure due to the presence of a large pulmonary embolus from the by-pass line.

Results

Twenty-five animals survived the technical procedure out of forty-one in which it was attempted. In the first twenty, seven only survived,

TABLE 1.

ORTHOTOPIC HOMOTRANSPLANTATION OF THE PORCINE LIVER (ANALYSIS OF RESULTS)
41 TRANSPLANTS ATTEMPTED

OPERATIVE DEATHS		DEATHS WITHIN 24 Hrs.		SURVIVORS		
SERIES						
1 - 20	21 - 41	1 - 20	21 - 41	TRANSPLANT No.	SURVIVAL TIME (Days)	TRANSPLANT DATE
13	3	3	6	T16	1·5	
				T21	3·5	
				T34	3·5	
				T29	4·0	
				T30	4·5	
				T37	4·5	
				T 7	5·5	
				T33	5·5	
				T40	6·0	
				T41	7·5	
				T23	8·0	
				T27	10·0	
				T36	21·0	
				T15	28·0	
TOTALS	16		9	T35	33·0	
				T 6		15-6-66

TABLE 2.

PATHOLOGICAL FEATURES AT POST-MORTEM OF 15 SURVIVORS

Survival time (days)	Transplant No.	Pathological findings at post-mortem
1·5	T16	Intraperitoneal Haemorrhage
3·5	T21	Liver Necrosis Thrombosed Arterial Supply
3·5	T34	G.I. Haemorrhage Oesophagogastric Ulceration
4·0	T29	Necrotic Gastric Mucous Membrane Rt. Pleural Effusion
4·5	T30	G.I. Haemorrhage Oesophagogastric Ulceration
4·5	T37	G.I. Haemorrhage Oesophagogastric Ulceration
5·5	T 7	Massive Pulmonary Embolus
5·5	T33	Patchy Liver Necrosis Multiple Peripheral Pulmonary Emboli
6·0	T40	G.I. Haemorrhage Oesophagogastric Ulceration
7·5	T41	G.I. Haemorrhage Oesophagogastric Ulceration
8·0	T23	No Definite Cause of Death Found ?Liver Rejection
10·0	T27	No Definite Cause of Death Found ?Liver Rejection
21·0	T36	G.I. Haemorrhage Oesophagogastric Ulceration
28·0	T15	Severe Unexplained Anaemia
33·0	T35	G.I. Haemorrhage. Oesophagogastric Ulceration

three of these dying within the ensuing 24 hours. This high mortality rate we attribute mainly to our own inexperience, to the initial use of relaxants which led to post-operative difficulties with respiration, to citrated blood which appeared to induce block of the cardiac conducting system and to not using heparin so that by-pass systems clotted and cardiac failure or pulmonary emboli ensued. In the second series of twenty-one transplants, eighteen survived although six of these died within 24 hours, mainly from haemorrhage, which has led us to reduce our heparin dose to its present level.

The survival times of the remaining sixteen animals range from 1-1/2 days to 9-1/2 months. (Table 1.) Two of the early deaths at 1-1/2 days and 3-1/2 days respectively were due to intraperitoneal haemorrhage and thrombosis of the aortic segment respectively and as such should be regarded as technical failures.

The fourteen animals left constitute the group from which information has been obtained. Thirteen of these survived for periods ranging from 3-1/2 days to 33 days and one remains alive at 9-1/2 months. The histological appearances of the livers of these animals together with biochemical data on liver function will be presented subsequently by Dr. Hunt, but the main causes of death found at autopsy are shown in Table 2. In only two of these animals, numbers 23 and 27, were the post-mortem findings such that no adequate cause of death was established and liver rejection alone might possibly be said to have been the main cause of death. Two other animals died at 5-1/2 days from pulmonary emboli, the sources of which were never detected and a further animal died at 28 days from a completely unexplained anaemia. The remaining eight animals all died of gastric complications, seven being due to oesophagogastric ulceration and severe gastrointestinal haemorrhage. In the eighth animal there was extensive and pulmonary collapse.

Oesophagogastric ulceration in the pig is not comparable to the gastrointestinal haemorrhage seen in canine liver transplants due to hepatic out-flow block, as it is a species hazard which occurs naturally in pigs.[2] The area of pig's stomach, just below the cardia, is predisposed to ulceration owing to the fact that a long tongue of squamous oesophageal epithelium which is devoid of protective mucus glands extends through the cardio-oesophageal sphinter into the body of the stomach. Although the exact cause of ulceration, as in man, remains unknown experimentally the lesions have been induced by histamine and by withholding feeding for three days.[3] In addition they have been produced by changes in fat content of the pig's diet.[4]

Unfortunately it now constitutes the single greatest danger to our transplanted animals and experiments are currently proceeding to see whether it can be controlled.

Summary

To summarize this the first of these three papers therefore; a series of sixteen pigs is presented in which orthotopic homotransplantation of the liver has been successfully accomplished. Two of these died at 1-1/2 and 3-1/2 days respectively from technical complications of the operation. The remaining fourteen animals have survived for periods ranging from 3-1/2 days to 9-1/2 months and one of these is alive today.

No definite pattern of liver rejection has been established and in all but two of the animals a cause of death was present at autopsy other than liver rejection. Four of the animals have survived for periods longer than 10 days, the survival times being 21, 28, 33 and 298 days respectively. Gastrointestinal haemorrhage due to oesophagogastric ulceration has been the main cause of death being directly responsible for seven of the deaths in this series of animals.

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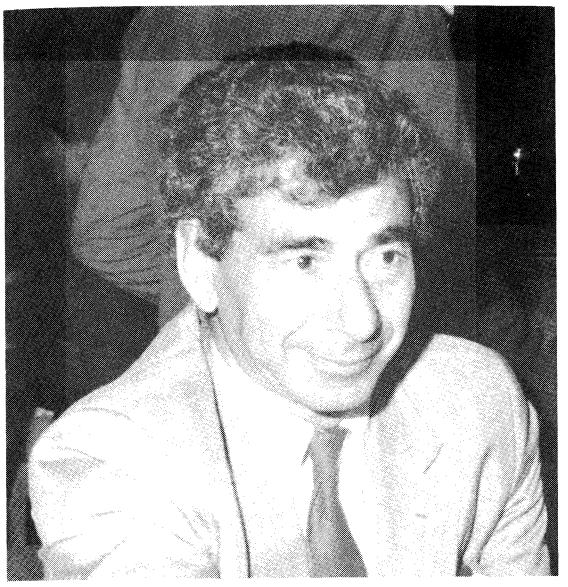

Sir Roy Calne's contributions to liver transplantation are so well represented in this collection of articles that further comments would be superfluous. In 1983, Calne published a book on his experience which was reviewed for the *Annals of Surgery 200:* 108, 1984 by one of the authors (Thomas E. Starzl) who concluded by saying "... the book provides insight and background about liver transplantation about which no new group could afford to be ignorant. It also has provided an accidental look at the creativity, courage in the face of terrible adversity, and vision of its author who is one of the great surgical scientists of the world."

Roy Calne, Professor and Chairman of the Department of Surgery at Cambridge University, extended observations on pig liver transplantation beyond those of the Paris and Bristol workers. In doing so, he speculated that the liver is a tolerogenic organ, a concept that still is being explored. Calne's group has used the pig model since for experiments in immunology, organ preservation and hepatic physiology. The work with pigs led to the opening of the historically important Cambridge-King's College (London) clinical liver unit which Calne has operated in collaboration with the hepatologist, Professor Roger Williams.

Observations of orthotopic liver transplantation in the pig

British Medical Journal, 2: 478-80, 1967

R. Y. Calne, H. J. O. White, D. E. Yoffa, R. R. Maginn, R. M. Binns, J. R. Samuel and V. P. Molina

Recent advances in renal transplantation permit excellent therapy in many patients, and surgeons are now turning to the possibility of clinical transplantation of other organs.

Initial liver transplantation experience was obtained in the dog with the use of methods previously described by Moore et al. (1960) and Starzl et al. (1960). Following the reports of Garnier et al. (1965), Cordier et al. (1966), and Terblanche et al. (1967) on orthotopic liver transplantation in the pig, we began on this animal in July 1966.

Materials and Methods

Nineteen liver transplants have been performed in large white pigs (20-30 kilograms) under halothane anaesthesia via an endotracheal tube. Donor and recipient were littermates in some cases; in others there was no relationship. The surgical technique was similar to that of Moore et al. and of Starzl et al.; however, minor modifications of the shunting procedure and arterial anastomosis were incorporated in certain experiments. In the first 11 operations, during the interruption of flow through the portal vein and inferior vena cava, blood from these two systems was shunted via catheters in the splenic and left femoral veins respectively joined to a T tube which connected to another catheter introduced into the left internal jugular vein. In the twelfth experiment two shunts were used — one from the splenic vein to the left jugular vein, the other from the right renal vein to the right jugular vein. The right kidney was removed. In the remaining seven operations catheters were inserted directly into the cut ends of the portal vein and inferior vena cava and connected respectively to the left and right jugular veins. In all cases the animals were heparinized while the shunts were open and the heparin was reversed with protamine at the end of the procedure. In the operations where the splenic vein was used, a splenectomy was performed.

In the first six experiments the donor liver was removed with the hepatic artery, coeliac artery, and a length of abdominal aorta which was anastomosed end-to-side to the abdominal aorta of the recipient. It was felt that this large vessel, with all but one of its outlets ligated, was haemodynamically unsatisfactory and its dissection added considerably to the time of the donor operation. Therefore in the subsequent experiments the coeliac artery with a Carrel patch of aorta was removed in continuity with the hepatic. In experiments 7-11, 13, 14 the coeliac artery of the donor was anstomosed to the side of the coeliac artery of the recipient. In experiment 12 the coeliac artery of the donor was anastomosed to the end of the right renal artery after the kidney had been removed. In the remaining five operations the coeliac artery was anstomosed with a Carrel patch directly to the abdominal aorta close to the origin of the coeliac artery of the recipient. In experiments 15 and 16 the coeliac artery was brought behind the stomach to the left side of the aorta. This resulted in tension, so in subsequent operations the coeliac artery was anastomosed direct to the right side of the aorta by the shortest route.

The anaesthetized donor pig was cooled to 33° C. in a bath of iced water prior to surgery. When the liver dissection was completed the animal was exsanguinated via the aorta. The blood was collected in ACD bags and used for transfusion into the recipient. The blood was neither grouped nor cross-matched. The liver was perfused via the portal vein with 2 litres of Ringer/lactate solution at 4° C. with a perfusion pressure of 1 metre of saline. During the operation and postoperatively, in addition to blood transfused from the donor, the recipient pig was given sodium bicarbonate and dextrose solution through an intravenous catheter in a suitable neck vein. One gramme of chloramphenicol was given intravenously during the operation and 1 megaunit of penicillin intramuscularly. The animals that survived the operation were given an ad lib. diet of commercial pig-food and water; they did not receive immunosuppressive agents. Venous samples of blood were taken for biochemical and haematological investigation at irregular intervals after operation. Postmortem examination was carried out on the animals. The thoracic and abdominal cavities were examined and sections of liver, lymph nodes, spleen in non-splenectomized animals, lungs, stomach, small intestine, and kidneys were examined histologically.

Results

The results are summarized in the Table. There were six operative deaths. The causes are shown in the Table, with the exception of experiment 10, where the cause of death was undetermined. This animal died at the end of the operation while the operative incision was being sutured. Five died within the first 36 hours. One of these animals had peritonitis and clot in the hepatic artery and donor section of aorta. In the other experiments the cause of death was not determined, but was presumed to be liver failure. In one of these cases the liver had been preserved for four and a half hours in ice before transplantation. Two animals died at three days. One

Orthotopic Liver Transplantation in the Pig

Experiment No.	Donor	Venous Shunts	Arterial Anastomosis	Survival	Microscopical Rejection	Comments
1	Littermate	Splenic and femoral to jugular	Aorta end-to-side abdominal aorta	29 hours	0	Cause of death undetermined
2	"	" "	" "	7 months	Minimal	Intestinal obstruction due to adhesions
3	"	" "	" "	Died on table		Venous shunt clotted
4	"	" "	" "	" " "		Air embolus via suprahepatic caval anastomosis
5	"	" "	" "	3 hours	0	Liver preserved 4½ hours in ice before transplantation
6	"	" "	" "	24 "	0	Peritonitis and thrombosis of hepatic artery and donor aorta
7	"	"	Coeliac end-to-side of coeliac	Died on table	0	Air embolus via jugular vein when removing catheter
8	"	" "	" "	7 days	+	Cystic duct inadvertently tied
9	Unrelated	" "	" "	Died on table	0	Pneumothorax
10	Littermate	" "	" "	" " "	0	Cause of death undetermined
11	"	" "	" "	14 days	+	Liver abscesses, peritonitis, necrosis of stomach, clot in left gastric artery
12	"	Splenic to jugular. Right renal to jugular	Coeliac end-to-end to right renal	9 "	+	Anaemia. Gastrointestinal haemorrhage
13	Unrelated	Portal to jugular. I.V.C. to jugular	Coeliac end-to-side to coeliac	30 hours	0	Cause of death undetermined
14	"	" "	" "	3 days	0	Thrombosed hepatic artery. Two intussusceptions
15	"	" "	Coeliac end-to-side to aorta	Died on table	0	Hypothermia
16	"	" "	" "	6 hours	0	Cause of death undetermined
17	Littermate	" "	" "	16 days	+	Anaemia. Gastrointestinal haemorrhage
18	Unrelated	" "	" "	12 "	+	" " "
19	Littermate	" "	" "	3 "	0	Cystic duct inadvertently tied "

had a thrombosed hepatic artery and two intussusceptions, the other had obstructive jaundice following inadvertent ligation of the cystic duct together with the common bile duct.

Five animals lived between 7 and 16 days. In each of these there was microscopical evidence of rejection. This consisted of focal necrosis of liver cells and round-cell infiltration of the portal triads. One of these animals died from obstructive jaundice due to ligation of the cystic duct with the common bile duct, another of multiple sepsis after necrosis of the anterior wall of the stomach due to embolization of the left gastric artery. The other three developed a syndrome of wasting and very severe anaemia due to bleeding from the gastrointestinal tract with melaena. The source of the haemorrhage was not determined with certainty in any of these animals, but it was thought to be from multiple superficial erosions of the gastric and duodenal mucosa.

The remaining animal lived with a transplant from its littermate for seven months after operation. Though it was extremely robust and ate well, it did not gain weight to the same extent as control animals and it grew more hair on its skin than normal. This animal died suddenly from intestinal obstruction due to adhesions. The liver parenchyma was remarkably normal at postmortem examination. There was a minimal increase of fibrous tissue in the portal triads with a light infiltration of mononuclear cells.

The chemical and haematological examinations of the blood were related to the clinical assessment and histological findings. Thus clinical jaundice was associated with a raised serum bilirubin, liver necrosis with raised S.G.O.T., S.G.P.T. and alkaline phosphatase, and melaena and pallor with low haemoglobin.

Discussion and Conclusions

In common with the French and Bristol workers, we have found the pig an extremely satisfactory experimental animal for orthotopic liver transplantation experiments. The pig withstands the surgery well and the liver appears to be rejected more slowly in the pig than in the dog, allowing more time for study of the animals, without necessity for immunosuppressive therapy. Halothane anaesthesia was effective; the animals were kept fairly light throughout the procedure and were usually on their feet within a few hours of the operation. In those animals surviving more than a week classical liver failure did not appear to be the cause of death. However, the gastrointestinal haemorrhage may well have been due to inadequate liver function, though the exact mechanism has not been determined. It will certainly be of interest to study the gastric secretion, metabolism of histamine, the haemodynamics of portal venous flow, and the composition and distribution of bile in these animlas. Peptic ulceration is a common complication of orthotopic liver transplantation in dogs. The Bristol workers have also observed gastrointestinal haemorrhage in pigs with orthotopic liver transplants and have demonstrated superficial mucosal erosions in the caridac portion of the stomach. A perforated duodenal ulcer was the cause of death of one of the pigs in the French experiments.

In our pig herd skin grafts are rejected aggressively when the donor and recipient are unrelated (Binn, 1967), and even in littermates rejection is the rule, though skin grafts may persist up to three weeks. The mechanism of acceptance of an orthotopic liver transplant in our longest-surviving animal at seven months and a similar animal of the Bristol group at nine months (Peacock, personal communication) has not been determined. Though splenectomy has little effect on the survival time of transplanted tissue in most species that have been studied, it is possible that splenectomy in pigs with liver transplants may influence the outcome of the transplant.

This early experience encourages us to pursue further studies with liver transplantation in the pig.

Summary

Our preliminary experience with orthotopic liver transplantation in the pig is reported. Of the 19 operations performed 13 animals survived the procedure and six lived for a week or more. One pig with an orthotopic liver transplant from a littermate lived for seven months after operation without any immunosuppressive therapy and died from intestinal obstruction, with minimal microscopical evidence of liver damage.

We wish to thank Dr. Marek Zakiewicz for the help he has given with the anaesthesia of the pigs, and we acknowledge the excellent technical assistance that we have received from the staff of the Department of Surgery, University of Cambridge, and the Agricultural Research Council at Babraham. We are most grateful to Dr. R. D. Keynes for providing facilities at the A.R.C., Babraham.

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Tom Marchioro was born in 1928, and grew up in Butte, Montana. His colorful life included jobs in mines, on construction gangs, and in odd jobs while working his way through college and medical school. He went to St. Louis University, School of Medicine and had his residency in St. Louis University, the Henry Ford Hospital in Detroit, and the University of Colorado. Many of his contributions to transplantation are distributed throughout this book.

This was the first systematic attempt to preserve organs by continuous hypothermic perfusion, using the cadaver as the perfusion chamber and providing flow and oxygenation with a mechanical heart/lung machine. The method was used clinically in the mid-1960's before brain death became a condition of organ donation. Cadavers with no heart beat could be cooled quickly and effectively with this technique. These first efforts at continuous perfusion formed the basis of the *in situ* cooling techniques that now are used universally as the initial step in modern-day, multiple-organ procurement (cf. Part III).

Today Marchioro is a Professor of Surgery at the University of Washington and Chief of Transplantation. Huntley, a gifted laboratory technician and administrator, runs a floral shop in Golden, Colorado. Waddell, who was Chairman of the Department of Surgery at the University of Colorado, "retired" to Silver City, New Mexico where he has an active surgical practice in the desert country where he grew up.

Extracorporeal perfusion for obtaining postmortem homografts

Surgery, 54: 900-11, 1963

T. L. Marchioro, R. T. Huntley, W. R. Waddell, T. E. Starzl

Successful organ homotransplantation is dependent upon the availability of satisfactory grafts. Paired organs, such as the kidney, may be obtained from living donors, whereas hepatic and cardiac grafts for human use can only be obtained post mortem. Cadaveric renal homografts have been largely unsuccessful to date.[3,11,12,19,21] The major cause of early failure was probably ischemic damage. This report describes an experimental method of procuring and temporarily preserving postmortem homografts of kidney and liver, and documents the clinical use of the technique in 4 cases.

METHODS

Thirty-two dogs weighing 9 to 20 kilograms were used. After the animals were anesthetized with 30 mg. per kilogram of pentobarbital intravenously, the femoral vessels on one side were exposed. Heparin, 3 mg. per kilogram, was injected intravenously, and aortic and inferior vena caval catheters were inserted through the femoral artery and vein (Fig. 1). The animals were then sacrificed with an overdose of pentobarbital. Respiratory arrest preceded cessation of hearbeat by 5 to 15 minutes. Perfusion was subsequently begun one to 22 minutes after the disappearance of palpable pulses, when it was certain that the animal was dead.

The catheters were connected to an extracorporeal perfusion system consisting of a disposable bubble oxygenator, a single De Bakey pump, and a heat exchanger (Fig. 1). Venous outflow was by gravity drainage. The oxygenator was primed with lactated Ringer's solution in all experiments except 2 hepatic transplants in which 5 percent dextrose in water was used. The perfusate was precooled to 15° C. by recirculation through the heat exchanger. One gram of procaine chloride was added to each liter of the perfusate. In prolonged perfusions, heparin 1.5 mg. per Kilogram, was added hourly. One third the original dose of procaine was added every 3 hours. With perfusions of 8 hours or more it was frequently necessary to add extra priming solution to the reservoir.

Pilot studies were first performed to determine suitable flow rates. The procedure was then standardized with initial flow rates of 40 to 60 ml. per kilogram per minute, and gradual reduction to 5 to 20 ml. per kilogram per minute, as the esophageal temperature fell below 20° C. Organ temperature was maintained between 12 and 15° C. thereafter by adjusting the temperature of the perfusate (Fig. 2).

All animals receiving renal homografts had bilateral nephrectomies. The donor left kidney was transferred to the right pelvis and the donor right kidney to the left pelvis of the host. Each recipient received only one kidney. The renal artery was anastomosed end to end to the iliac artery of the recipient and the renal vein end to side to the iliac vein. In animals receiving hepatic homografts, the liver was transplanted orthotopically after recipient hepatectomy.[17,18] Postoperatively, azathioprine (Burroughs-

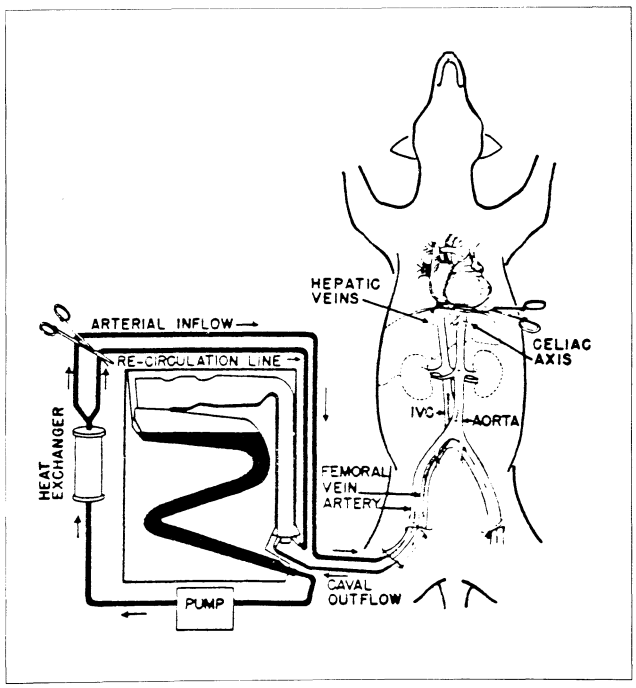

Fig. 1.— Technique of extracorporeal cadaver perfusion.

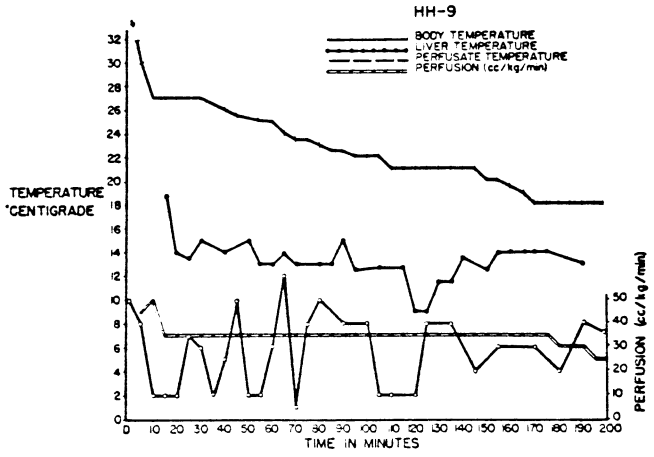

Fig. 2.— Cooling curves obtained during cadaver perfusion for a liver homograft. Note the rapid response of liver temperature to changes in perfusate temperature.

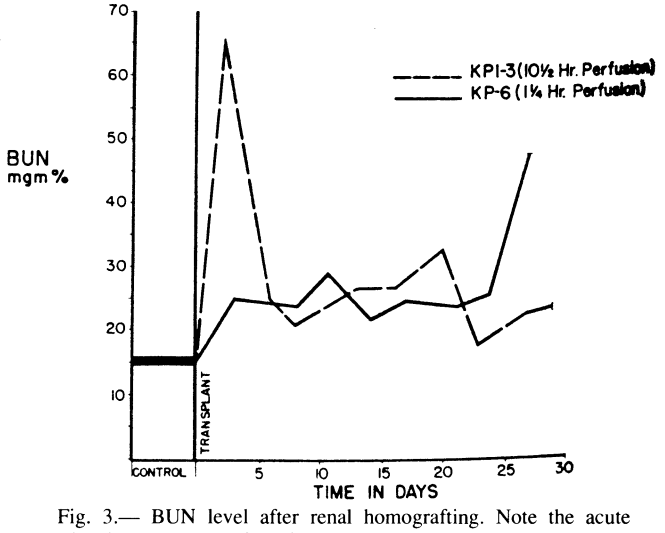

Fig. 3.— BUN level after renal homografting. Note the acute azotemia with the homograft perfused for 10-1/2 hours. Early azotemia is minimal in the animal receiving a kidney perfused for 1-1/4 hours.

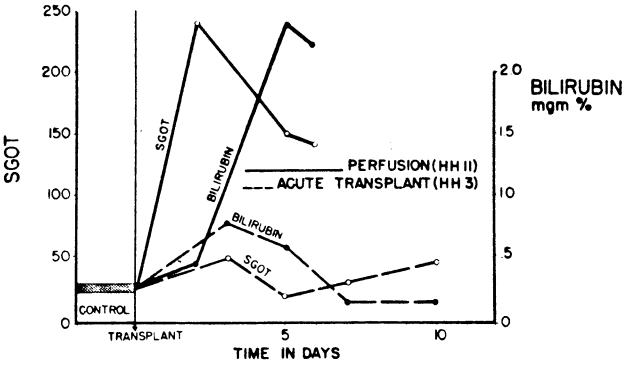

Fig. 4.— Patterns of injury in dogs observed following transplantation of a perfused hepatic homograft, and of a liver obtained after sacrifice of a living donor. Total time from death to reimplantation for the perfused organ was 140 minutes as compared to 55 minutes for the graft transplanted immediately after sacrifice (acute transplant).

Wellcome 57-322, Imuran) was administered to prevent rejection. Results were judged by function of the grafts and by subsequent histologic studies.

In clinical use, insertion of the catheters was not done until the patient's death had been verified by the attending physician. In these instances, heparin was added to the perfusate in an amount equal to 3 mg. per kilogram of body weight of the proposed donor. Total time to make the necessary incisions, insert the catheters, and begin perfusion has not exceeded 15 minutes from death in 4 clinical applications.

RESULTS

Pressure-flow studies. Blood pressure monitoring was found to be of no value in determining flow adjustments. In order to maintain an arterial pressure of 50 to 70 mm. Hg, flow rates as great as 600 ml. per kilogram per minute were required, resulting in acute swelling or even rupture of the various organs. Flow rates were therefore arbitrarily limited to 40 to 60 ml. per kilogram per minute, and gradually reduced to 5 to 20 ml. per kilogram per minute as the temperature fell to 20° C. Under these conditions of perfusion, arterial pressures never exceeded 20 mm. Hg and were usually unobtainable. Despite the low flow perfusion, cooling was relatively rapid (Fig. 2).

Degree of cooling. Five animals were cooled to 2 to 10° C. by the low-flow technique. These temperatures were usually reached in less than

Table I. *Renal homografts*

Dog no.	*Perfusion started post mortem (min.)*	*Flow rates (ml./Kg.) High-low*	*Time to reach 15° C. (min.)*	*Total time from death to revascularization (hr., min.)*	*Urine flow started*	*Survival post transplantation (days)*	*Cause of death*
1	5	40-20	45 (to 21° C.)	2, 15	Immediately	36	Gastrointestinal bleeding
2	5	60-20	24	4, 20	Immediately	31	Pneumonia
3	4	19.5- 4.5	90	4, 42	Immediately	10	Intussusception
4	12	32-15	6	3, 31	Second day P.O.	10	Pneumonia
5	8	30- 7	10	6, 15	Immediately	10	Pneumonia
6	8	30- 7	10	6, 20	Immediately	16	Pneumonia
7	5	40-15	70	13, 6	Immediately	13	Pneumonia
8	5	40-15	70	14, 33	Immediately	11	Pulmonary emboli
9	5	30-15	45	11, 12	Immediately	52	Pneumonia
10	5	30-15	45	14, 23	Immediately	23	Pneumonia
11	5	50-15	22	13, 27	Immediately	7	Pneumonia

Table II. *Hepatic homografts*

Dog.. no.	*Perfusion started post mortem (min.)*	*Flow rates (ml./Kg.) high-low*	*Time to reach 15° C. (min.)*	*Total time from death to revascularization (hr., min.)*	*Survival post transplant*	*Cause of death*
1	7	60-20	24	2, 3	No	Hemorrhage
2	4			3, 54	No	Hemorrhage
3	5	50-25	18	4, 22	No	Hemorrhage
4	Immediate	15.5- 2.5	17	3,	No	Hemorrhage
5	7	32-15	28	2, 18	4 days	Bile peritonitis
6	8	33- 3	22	3, 38	No	Hemorrhage
7	4	195-45	90	3, 42	5 days	Intussusception
8	Immediate	29-12	19	8, 18	2 days	Hemorrhage
9	5	40-15	8	4, 55	3 days	Hemorrhage
10	22	27-12	10	4, 47	24 hr.	Hemorrhage

45 minutes. Renal homotransplantation was then performed. Immediately after revascularization, the organs assumed a mottled pink and blue hue with islands of cyanotic tissue interspersed among areas of normal parenchyma. During the next hour, the kidney gradually became grossly normal in appearance. Nevertheless, none of the grafts produced urine and all 5 animals died with uremia within 2 to 3 days.

When temperatures were kept at 15° C. or above, such mottling of the kidney did not occur, and urine production was prompt. In the definitive series to be described, temperatures were, therefore, not allowed to fall below 15° C.

Renal homografts. Eleven renal homografts were performed after cadaver perfusion of 1 to 14 hours (Table I). In 5 of the 11 cadavers, perfusion was carried out for 11 or more hours. Perfusion time for the entire series averaged 7 hours and 33 minutes. Revascularization in the recipient dog was usually completed within 30 minutes after removal of the kidney from the perfused cadaver.

Ten of the 11 animals produced urine immediately and maintained good urinary volumes until death. The eleventh animal was anuric for 2 days, with subsequent diuresis.

Renal function was assessed by urine volumes and blood urea

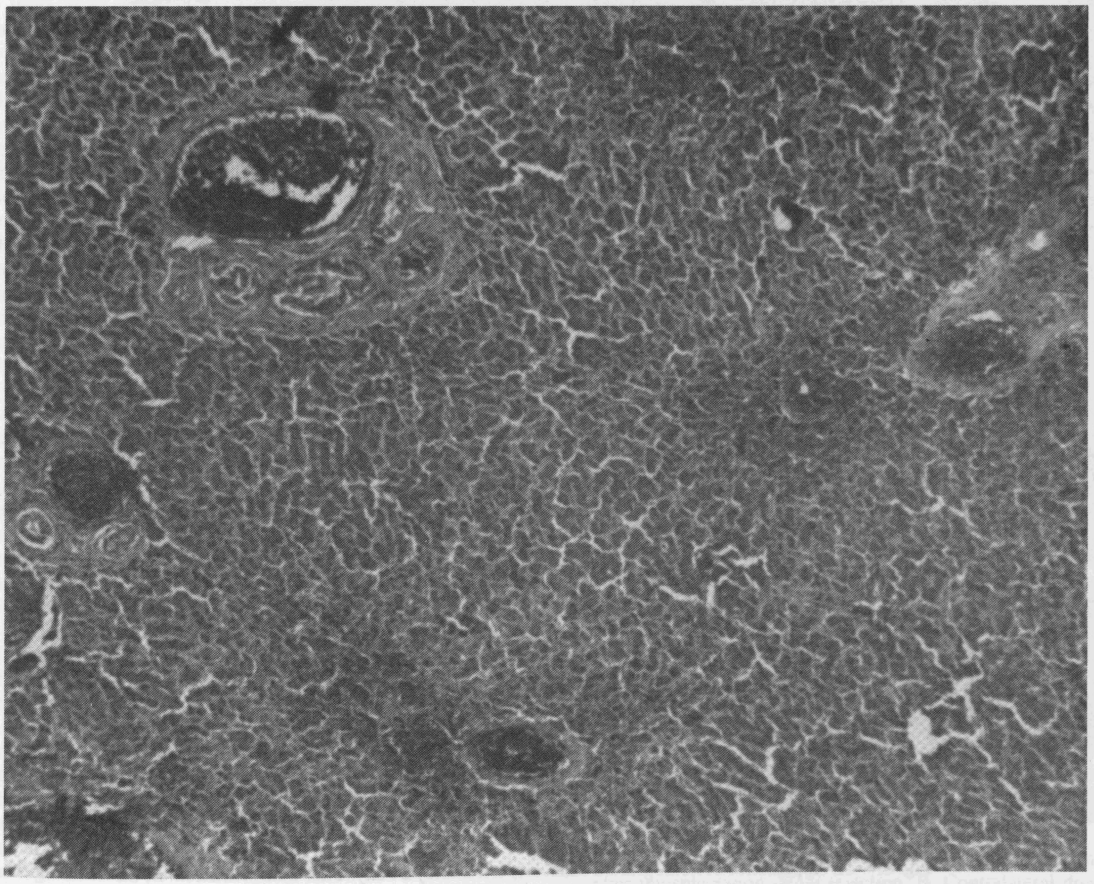

Fig. 5.— Typical injury observed in livers perfused for more than 2 hours. Note the marked centrilobular congestion and parenchymal necrosis (x 32).

nitrogen (BUN) determinations. The animals receiving kidneys which had been perfused for 6 hours or more showed sharp postoperative rises in the BUN (Fig. 3). This subsequently returned toward normal. With perfusion of less than 6 hours, this pattern of early azotemia was not prominent (Fig. 3).

Survival ranged from 7 to 52 days (Table I). Mean survival time was 19.9 days. Death most commonly resulted from pneumonitis, with 8 dogs dying of this complication. Six of the animals were uremic at the time of death. Histologic sections of the transplanted kidneys removed post mortem showed varying degrees of mononuclear cell infiltrates with minimal evidence of rejection. In no instance was there evidence of histologic renal damage which could be directly attributed to the perfusion.

Hepatic homografts. Hepatic homografts were transplanted in 10 animals after perfusions ranging from 71 to 416 minutes (Table II). Revascularization after removal of the liver required an additional average time of 72 minutes. Hepatic function after transplantation was inferred from the ability of the animal to awaken from pentobarbital anesthesia and survive without supplemental glucose therapy.

Five animals survived the immediate postoperative period. Severe hepatic damage was observed in all, as evidenced by sharp rises in serum glutamic oxalacetic acid transaminase (SGOT) and bilirubin levels (Fig. 4). Three of these animals later died of hemorrhage at 24 hours, 2 days, and 3 days postoperatively.

Consistent clotting defects were encountered in all animals. Increased fibrinolysin, decreased plasma fibrinogen contents, and increased thrombin times were uniformly observed. The significance of the clotting defects is reflected in the high death rate from hemorrhage (Table II). Five animals died from this cause either on the operating table or immediately postoperatively; another 3 which survived the procedure had extensive hemoperitoneum at autopsy. The incidence of fatal hemorrhage increased with increased length of perfusion of the donor liver. Six of the 8 animals that died from this cause had received hepatic homografts from donors that had been perfused in excess of 2 hours (Table II).

Histologic sections taken post mortem correlated well with the clinical observations. All livers showed moderate to marked centrilobular congestion and hepatic cell necrosis (Fig. 5) without evidence of rejection.

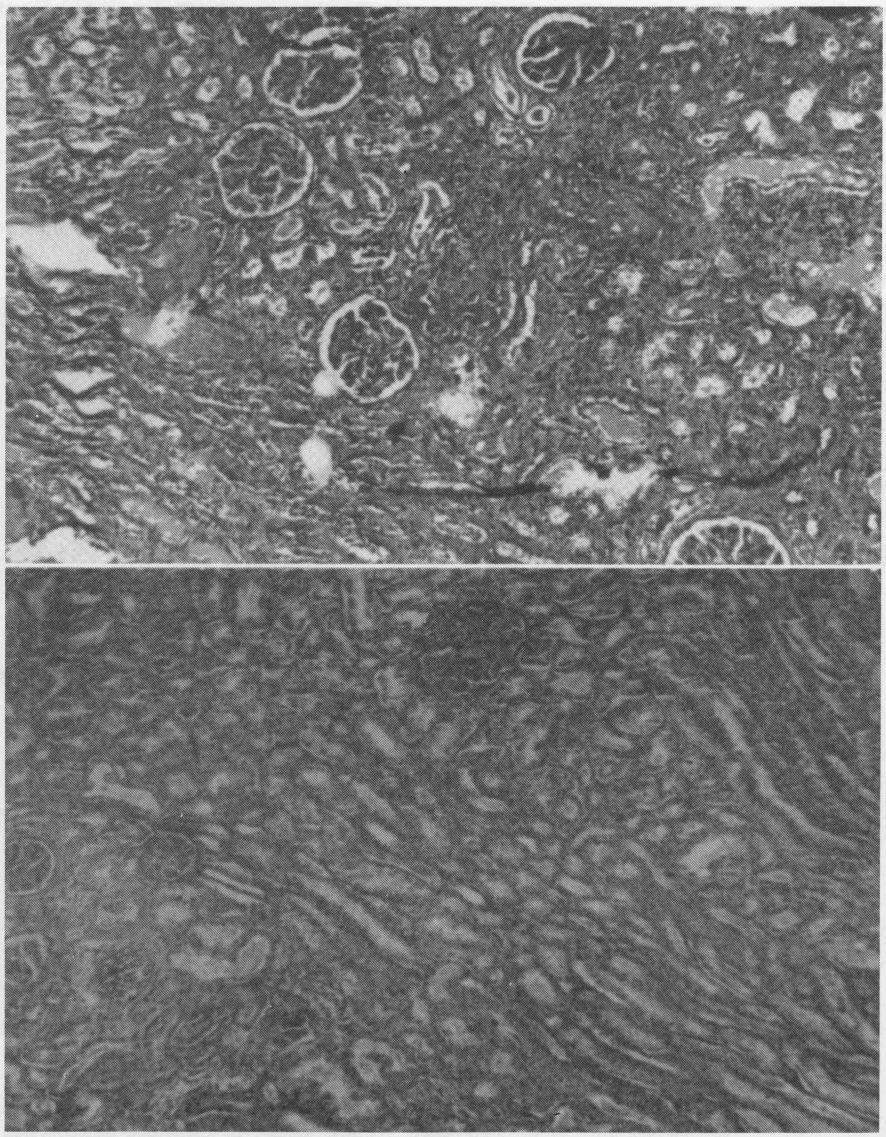

Fig. 6.— A, Appearance of homografted cadaveric left kidney 24 days after transplantation. B, Cadaveric contralateral kidney immediately after cessation of perfusion (x32).

Clincal use of cadaveric renal homografts. The first homograft was obtained from a patient who was thought to have a brain tumor, but who was subsequently shown at autopsy to have died of acute bacterial endocarditis. His blood pressure was 60 mm. Hg or less for 4 hours prior to death. Cadaver perfusion was not satisfactory, the maximal flow rates being 15 to 20 ml. per kilogram per minute. Revascularization of the kidney in the recipient bed was accomplished 106 minutes after death. The homograft functioned for 2 weeks postoperatively, although at reduced efficiency. The BUN fell from 176 to 119 mg. percent, and then rose again after 12 days. Death of the host from sepsis occurred on the twenty-fourth day after transplantation. Histologic sections of the transplanted kidney showed severe interstitial hemorrhage and the presence of proteinaceous material in the tubules. There was minimal mononuclear infiltrate (Fig. 6). Sections obtained from the opposite donor kidney after perfusion revealed normal architecture (Fig. 6). There was no evidence that septic emboli in the transplanted tissue contributed to the unfavorable outcome.

Postmortem hypothermic perfusion in the second case failed within a few minutes after its inception. The patient, who died of subarachnoid hemorrhage, was severly dehydrated terminally, and probably had a low blood volume. Adequate venous return could not be obtained. The kidney was revascularized in the recipient bed 124 minutes after death. Renal function did not return. Twelve days later, the graft was removed, after it had ruptured following relatively minor trauma to the recipient patient in a fall from bed. Histologic sections showed typical rejection (Fig. 7).

Clinical use of two cadaveric hepatic homografts. The first organ was obtained from a 3-year-old child who died in the operating room during resection of a medulloblastoma. The recipient was another 3-year-old child with terminal biliary cirrhosis secondary to congenital atresia of the bile ducts. The time from death to the institution of extracorporeal perfusion was 15 minutes. For the preceding 30 minutes, open cardiac massage was performed. Initial perfusion flow rates were 30 to 50 ml. per kilogram per minute and cooling proceeded rapidly. Perfusion was then reduced to 10 to 20 ml. per kilogram per minute. After 2 hours, venous return began to decline and during the next 110 minutes perfusion ceased altogether. The temperature of the liver at the time of perfusion failure was 15° C. After an additional 45 minutes, the liver was removed. Orthotopic revascularization in the recipient took 85 minutes.

Following restoration of blood supply to the donor liver, a bleeding diathesis developed with hypofibrinogenemia and fibrinolysis. Despite the administration of fresh whole blood, fibrinogen, and epsilon-amino-caproic acid, the hemorrhage could not be controlled. Histologic sections of the transplanted liver showed well-advanced, non-specific autolysis which could not be attributed to ordinary postmortem changes (Fig. 8).

The second liver homograft was obtained from a 55-year-old man who died from a cerebral glioma. The recipient was a 47-year-old man in whom a primary hepatoma had been found at operation one week earlier. Several measures were taken to shorten the necessary time for and to improve the quality of perfusion. The recipient was prepared for the homograft by a preliminary operation, 24 hours before definitive transplantation, at which time his own liver was freed of all attachments except the bile ducts and vessels. Intravenous infusions of glucose were given to the prospective donor in order to maintain hepatic glycogen. Immediately after death had been certified, acute expansion of the intravascular volume was achieved by forced transfusion of whole blood and plasma. Finally, the thoracic aorta was occluded just above the diaphragm (Fig. 1).

Perfusion at 40 ml. per kilogram per minute was begun 5 minutes after death, and gradually reduced to 20 ml. per kilogram per minute during the next 45 minutes, during which time the body temperature fell to 15° C. Dissection and extirpation of the donor organ required 94 minutes. After removal, 500 c.c. of cooled lactated Ringer's solution was used to wash out residual blood. The liver was then carried to the recipient operating room and revascularized in the ensuing 63 minutes. Bile flow through the transected donor common duct was noted shortly thereafter. Epsylon-amino-caproic acid, fibrinogen, and fresh blood were administered during transplantation. Bleeding was not a major problem.

In spite of the success of perfusion and the short time from death of the donor to revascularization of the liver in the host, evidence of hepatic damage was observed in the first few postoperative days with rises of SGOT to as high as 1,150 units and an early rise of bilirubin to 12 mg. percent. Liver function tests gradually returned toward normal until death occurred on the twenty-second postoperative day as a result of multiple pulmonary emboli. Histologic sections of the liver showed preservation of architecture (Fig. 9). There was some increased fibrosis and bile stasis. No damage due to perfusion was seen, nor was there unequivocal microscopic evidence of rejection.

DISCUSSION

The beneficial effects of hypothermia in preventing ischemic damage to tissue have been documented for several organs, including kidney,[5,6,8,10,15] liver,[1,4,13,16,17] lungs,[2] and heart.[9] Perfusion of isolated organs with

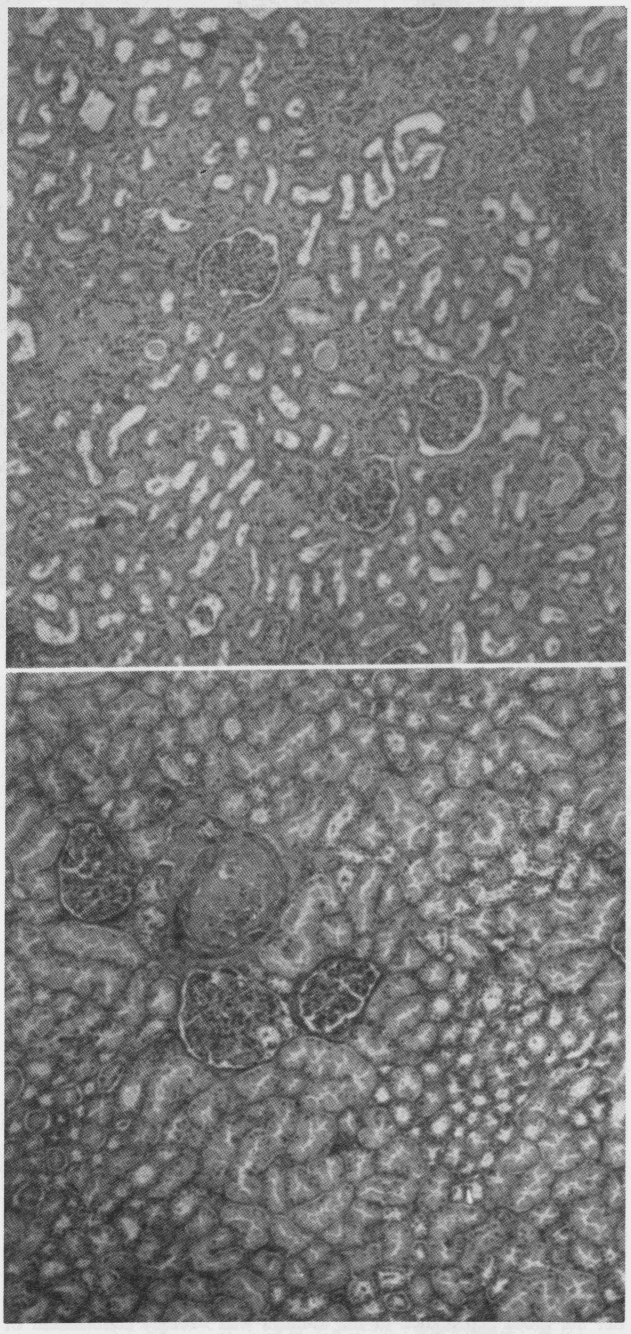

Fig. 7.— A, Homografted cadaveric right kidney removed 12 days after transplantation. Note rejection. B, Contralateral donor kidney obtained at conclusion of perfusion (x32).

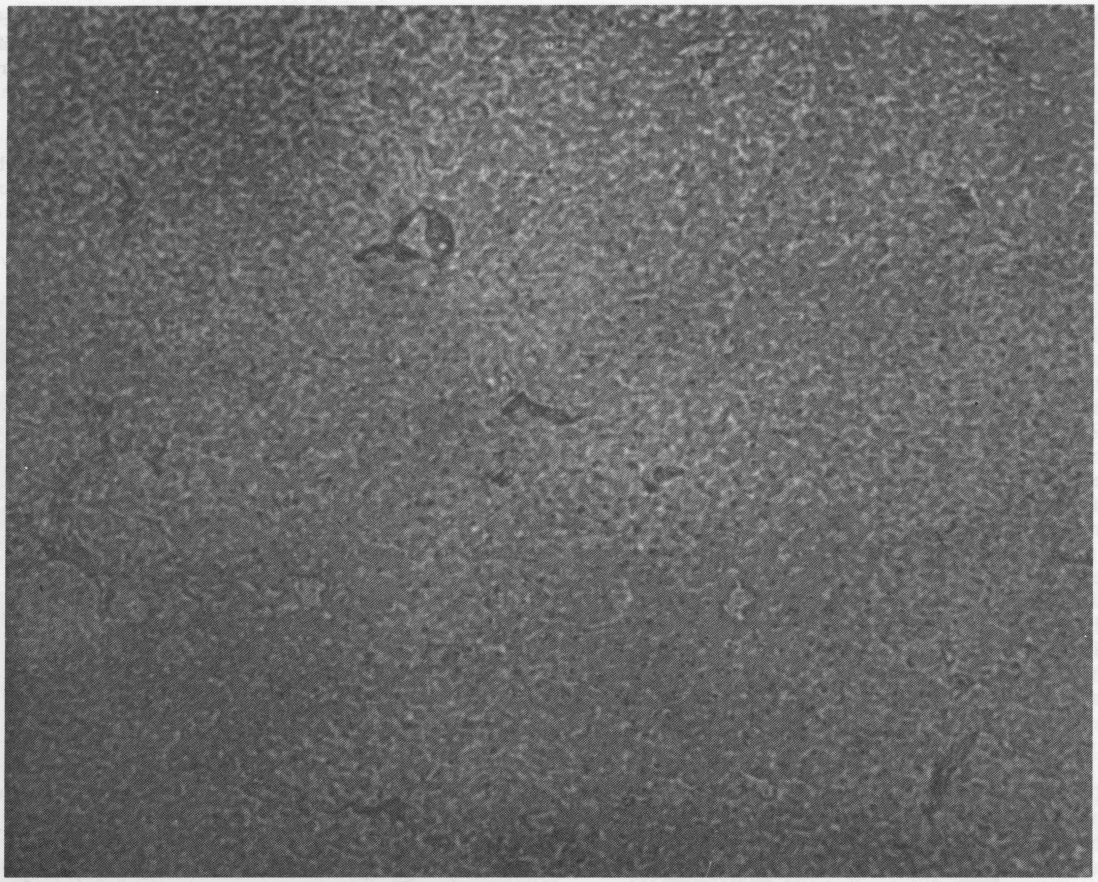

Fig. 8.— Photomicrograph of first clinical hepatic homograft. The patient died on the operating table. Note advanced hepatic cell necrosis (x32).

various solutions had also been shown to prolong function. The technique described here incorporates the advantages of both hypothermia and perfusion. The practicality of the method is considerably increased by the use of the electrolyte- or glucose-primed disposable bubble oxygenator, thereby eliminating the need for blood typing. The resultant hemodilution reduces viscosity[14] and promotes increased renal blood flow.[7] Moreover, oxygen is available to the tissues in quantities equal to those obtained from whole blood.[20] The favorable effect of procaine on renal perfusion at low temperatures has been demonstrated by Kiser and his colleagues.[8]

Extracorporeal perfusion in cadavers differs from that in the living patient. Serious organ damage resulted from overperfusion when it was attempted to maintain arterial pressures which would be clinically acceptable. Apparently there is a rapid and complete loss of vascular tone shortly after death, making the maintenance of significant arterial pressure impossible except with exorbitant flow rates. The use of low flows in combination with hypothermia provides some protection against hydrostatic damage to the organs from the pump system. While low-flow perfusion has metabolic disadvantages, it has appeared to be satisfactory for short-term preservation.

Although others have been able to obtain satisfactory renal function after storage of kidneys at low temperatures (1 to 4° C.) for as long as 8 hours,[8,15] we were unable to do so with continuous perfusion at these temperatures. Renal homografts cooled below 10° C. failed to function. When temperature was controlled at 15° C. all renal homografts were functional (Table I). The use of extremely low temperatures with continuous perfusion appears to be harmful. The mechanism of injury is unknown, but the gross appearance of the kidneys was strongly reminiscent of frostbitten extremities.

Although satisfactory cadaveric renal homografts have been consistently obtained with the use of the low-flow perfusion at temperatures of 15° C. for as long as 14 hours after death, there are limitations to the method. Kidneys perfused for longer than 6 hours showed temporarily depressed renal function. The liver has been shown to be even more susceptible to postmortem injury. This is evidenced by the high failure rate in the animals perfused over 2 hours (Table II). Even with perfusion for less than 2 hours, serious hepatic damage occurs, as shown by elevation in SGOT and bilirubin levels, and by the development of coagulation defects.

Comparing the animal results with the clinical material, it is apparent that success depends as much on the terminal course of the donor as it does on the perfusion. Renal homografts obtained from animals sacrificed with normal cardiovascular dynamics have been uniformly successful. Cadaveric perfusion in such dogs requires no special precautions. In contrast, kidneys and livers obtained from patients who have had a protracted terminal course are variably damaged before death, resulting in unpredictable function of the homografts. In addition, difficulties with perfusion have been encountered, apparently due to a reduced blood volume, inasmuch as transfusion of the cadaver partially corrects this problem. If flows are still inadequate, selective perfusion of the lower half of the corpse has been obtained by clamping the lower thoracic aorta. Despite these adverse factors, it is encouraging to note the fact that functional hepatic and renal homografts have, nevertheless, been obtained for clinical use.

Other organs, including lung and heart, have been successfully transplanted in animals, from living donors. Homografts of heart and lung, to be clinically useful, must be obtainable from other sources. The technique described should be applicable with some modifications. It will be necessary to decompress the pulmonary circuit with left atrial or ventricular vents to prevent damage to the lung or overdistention of the left ventricle.

SUMMARY

Functional renal homografts have been obtained as long as 14 hours post mortem by a technique of extracorporeal cadaver perfusion. The

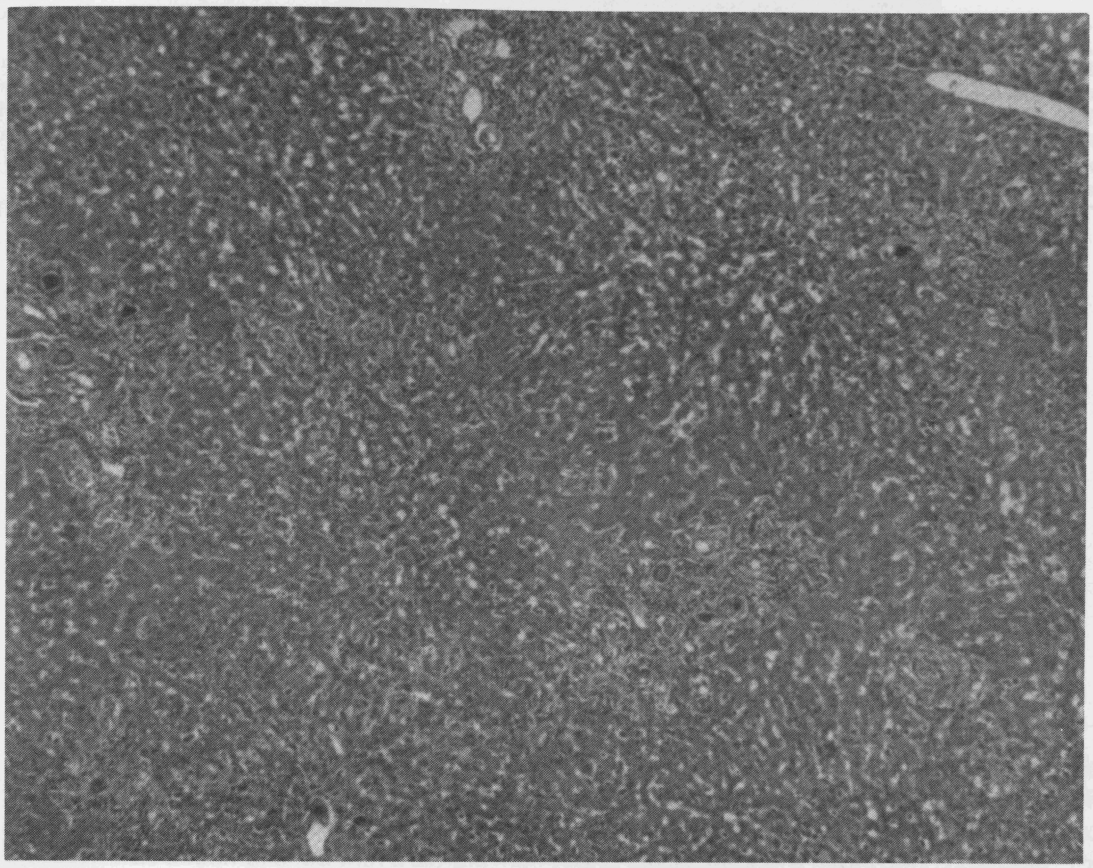

Fig. 9.— Photomicrograph of liver from the second clinical hepatic transplant, obtained at autopsy 22 days after transplantation. Note preservation of hepatic architecture. Moderate periportal fibrosis and bile stasis are present (x32).

method has also given satisfactory liver homografts after perfusion up to 2 hours. Details of the technique and experimental and clinical applications are described. Analysis of the data indicates that optimal perfusion times for renal homografts should not exceed 6 hours. For the liver, 2 hours appears to be the maximum tolerable period of perfusion.

For clinical application, certain adjuvant measures are important. Perfusion failure has been encountered in human cadavers, presumably as a result of the presence of a reduced blood volume. This can be prevented by acute postmortem expansion of the intravascular volume with compatible whole blood or plasma. Proper donor selection is as important a factor as the method used for preservation. Indirect evidence is cited that premortem tissue injury in protracted terminal disease may be a greater deterrent to success than the parenchymal damage which occurs during postmortem perfusion.

The extension of this technique for the procurement of other organs such as heart and lung is discussed.

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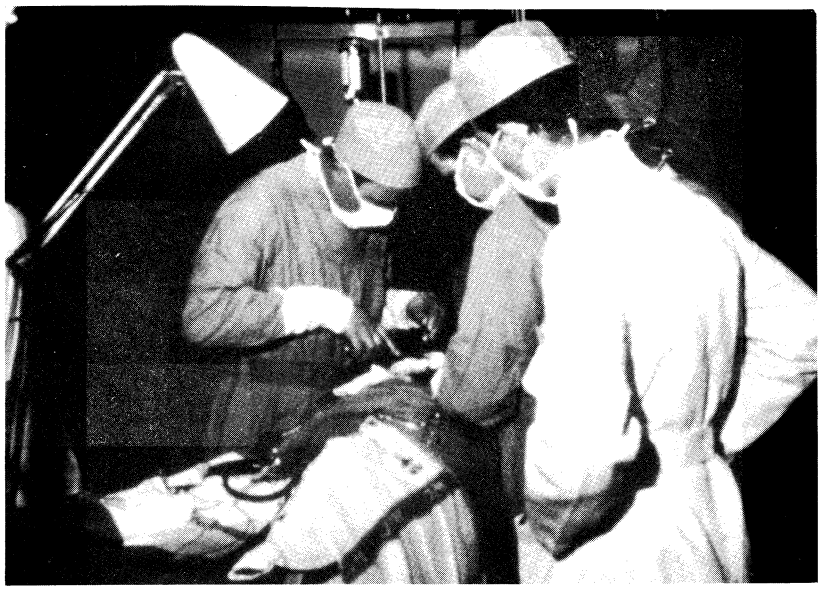

Lawrence Brettschneider, M.D., in 1967, working on a preservation experiment.

Brettschneider was a young naval officer detached to the University of Colorado in 1966-8. For the first time, he showed that the liver could be perfused continuously *ex vivo* and orthotopically transplanted successfully as long as one or two days later. The perfusate contained whole blood; oxygenation was accomplished with a simple membrane oxygenator in a hyperbaric chamber. The method was used clinically on several occasions but eventually abandoned because of its complexity and potential dangers. These extraordinarily heavy hyperbaric chambers were manufactured by the Bethlehem Steel Corporation in Pennsylvania. Brettschneider died in 1978.

The use of combined preservation techniques for extended storage of orthotopic liver homografts

Surgery, Gynecology & Obstetrics, 126: 263-74, 1968

Lawrence Brettschneider, Pierre M. Daloze, Claude Huguet, Kenneth A. Porter, Carl G. Groth, Noboru Kashiwagi, David E. Hutchison and Thomas E. Starzl

During the 12 years since homotransplantation of the whole canine liver was first described by Welch, there have been reports of several techniques of hepatic homograft preservation in dogs. Goodrich and his colleagues showed that livers removed at normal temperatures became unsuitable for transplantation within 20 or 30 minutes. The first protective device used in our laboratories (15) and by Moore and his associates was hypothermia, induced first by whole body cooling of the donor to 30 degrees C. and then perfusing the excised liver with a chilled electrolyte solution. With a fall in the hepatic core temperature to approximately 15 degrees C., these organs could support the life of recipient dogs if revascularized as orthotopic homografts within 2 hours. After longer times, there was a high rate of acute failure due to outflow block of the transplants, a hemorrhagic diathesis, and acute liver failure. Almost identical conclusions about the efficacy of this simple approach were reached by Ono and his co-workers in experiments which did not involve homotransplantation.

Subsequent efforts to extend the acceptable storage time have been disappointing. In dogs, Marchioro and his associates reported a method of hypothermic cadaveric perfusion with the use of an extracorporeal heart-lung apparatus into which a heat exchanger was incorporated. Either the entire corpse or the lower half of the dog was perfused. Orthotopic homotransplantation was performed with livers removed from 2 to 8 hours after the sacrifice of the donors. Eight of the 10 recipients which were treated with azathioprine survived operation, but all died within 1 to 5 days thereafter. Mikaeloff and Kestens and their associates used a similar principle in which hypothermic perfusion in situ was limited to the liver. They were able to obtain long term recipient survival after homotransplantation of canine livers removed as long as 6 hours after death. Their technique was an application of a method described several years earlier by Kestens and McDermott.

More complex methods have been tried. Brown and his colleagues have evaluated a combination of freezing to -6 degrees C., immersion into a dimethylsulfoxide or glycerol bath, and dehydration. After 1 to 5 days, the organs were viable but severely damaged and apparently incapable of supporting life as orthotopic homografts. When Moss and his co-workers cooled liver to -20 to -60 degrees C. for 1 to 14 days without dehydration, there was almost no function after the homografts were transplanted to the pelvis. Furthermore, all of the recipients died in 6 hours or less.

Recently, there have been 2 reports of conservation of hepatic homografts for 8 to 24 hours, a combination of perfusion, hypothermia, and hyperbaric oxygenation being used. Slapak and his associates placed puppy livers preserved in this way in the neck of adult recipients and found that bile was produced in 8 of 19 experiments. Their perfusing fluid did not contain red blood cells. In a preliminary report from our laboratory (3), chronic survival was described of adult canine recipients that received orthotopic hepatic homografts which had been preserved for almost a day after sacrifice of the donor. In these experiments, the perfusate contained diluted blood. As will be documented, further experience has shown that a significant rate of survival can be attained with such conserved organs if attention is paid to several important details.

METHODS

Organs were obtained from nonrelated mongrel donors and transplanted as orthotopic homografts by a modification of previously described techniques (Fig. 1). Anesthesia was with pentobarbital sodium, supplemented with phencyclidine hydrochloride. The recipients were 12 to 23 kilograms in weight.

Each of the donor dogs, which were of approximately matching size, was cooled to 30 to 34 degrees C. in an ice bath and sacrificed for removal of the liver. Just before and during donor hepatectomy, the liver was further cooled by an intraportal infusion with a balanced electrolyte solution, which had been buffered to pH 7.45 and chilled to 2 degrees C. The constituents included 2.5 grams per hundred milliliters low molecular weight dextran, 150 milligrams per hundred milliliters glucose, 2 milliequivalents per liter magnesium sulfate, and 50 milligrams per liter procaine. A total of 1,000 to 2,000 milliliters was used.

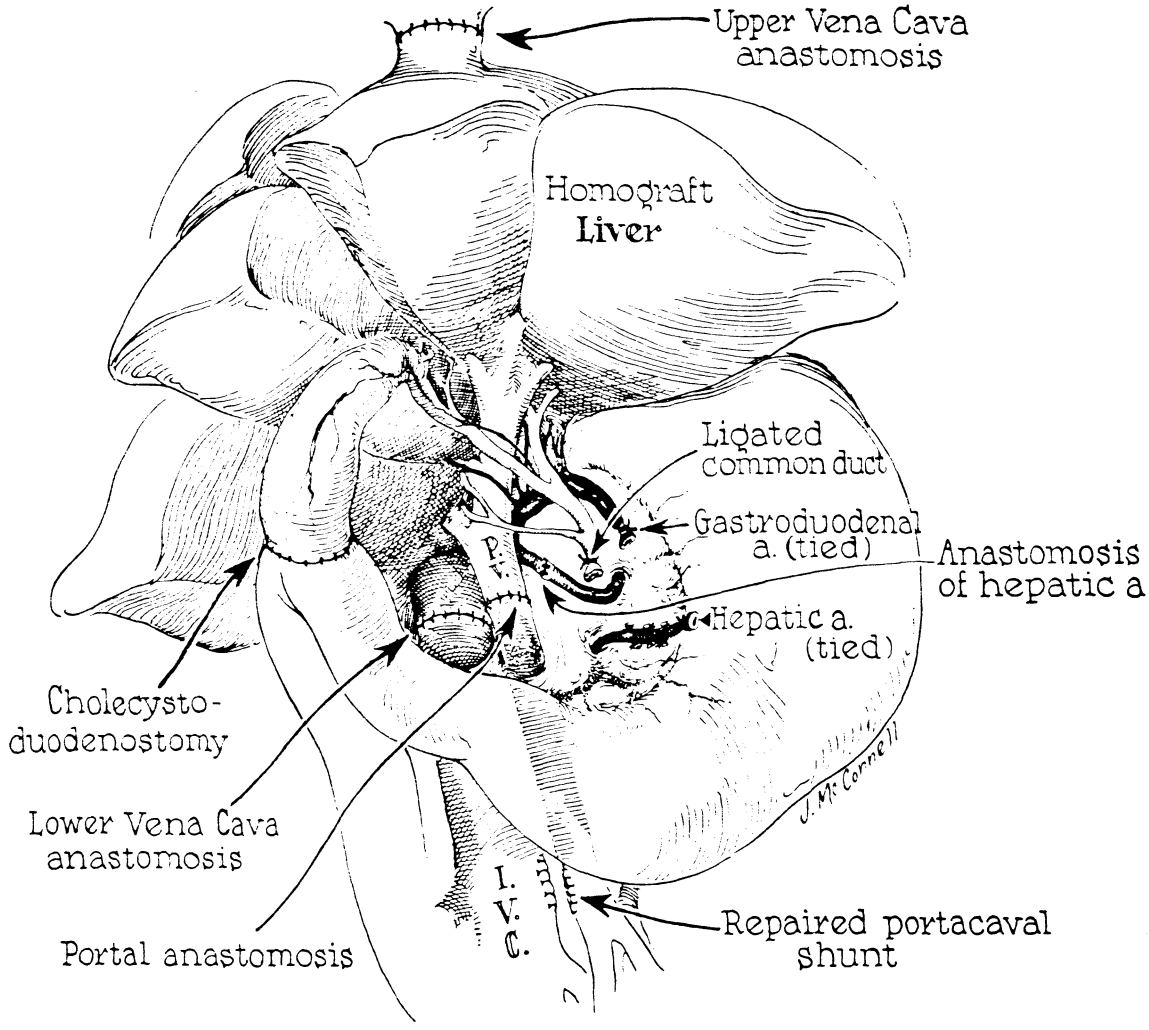

Fig. 1.— Technique of orthotopic liver transplantation. The arterial supply is derived from the proximal part of the common hepatic artery by an end-to-end anastomosis to the proximal part of the recipient hepatic artery. The distal portion of the recipient artery and its inferiorly directed branches are left undisturbed. Failure to do so may result in duodenal necrosis.

Homografts for control experiments were transplanted as quickly as possible, revascularization in the recipient usually being completed within an hour. Organs which were to be preserved for longer periods had cannulas tied into the aorta and portal vein (Fig. 2, inset). The homografts were inserted into a hyperbaric chamber which was refrigerated at 4 degrees C. Other aspects of the preservation technique which included perfusion and control of oxygen pressure were varied as will be defined in the different experimental groups.

During the subsequent transplantation, all dogs were given whole blood and 5 per cent glucose in lactated Ringer's solution. In each experiment, at least 500 milliliters of each were used, but more was given when needed. In addition, the recipients of preserved livers received 100 milligrams per kilogram epsilon aminocaproic acid, 1 milligram per kilogram protamine sulfate, and 20 milliequivalents sodium bicarbonate at the time of homograft revascularization. Repeat doses of these drugs were very often necessary.

For all recipient dogs, immunosuppression was provided with intramuscularly administered antilymphocyte globulin and orally administered azathioprine and prednisone. The antilymphocyte globulin was usually started several days in advance of operation and continued daily for several weeks after. Azathioprine therapy was begun at the time of transplantation and given indefinitely in the maximum doses per day which did not cause leukopenia. A 1 week course of prednisone was administered in progressively diminishing daily doses which were started at 3 milligrams per kilogram on the day of operation.

Postoperative liver function was monitored with frequent measures of serum bilirubin, serum alkaline phosphatase, total protein, serum glutamic oxalacetic transaminase, and serum glutamic pyruvic transaminase. In the event of death, autopsies were performed with particular attention being given to the state of the vascular anastomoses. Homograft tissue was fixed in 10 per cent formalin for eventual study with light microscopy.

Group 1, control. This group consisted of 12 dogs having homotransplantation without extended preservation.

Group 2, hyperbaric oxygenation and perfusion with diluted blood. Twenty-six homografts were placed in the hyperbaric chamber and continuously perfused with fresh homologous blood mixed with an equal volume of a solution identical to that employed for infusion, except that it contained 100 milligrams per liter heparin and 5 instead of 2.5 grams per hundred milliliters low molecular weight dextran. During preservation, the pH of the perfusate was frequently measured. If this fell below 7.2, it was corrected with sodium bicarbonate. A simple baffle runoff from the organ

TABLE I.—RESULTS OF LIVER TESTS ON THE FIRST DAY AFTER TRANSPLANTATION COMPARED TO THOSE IN 40 NORMAL DONORS

Group	*No.*	*Preservation time, hrs.*	*Serum glutamic oxalacetic transaminase*	*Serum glutamic pyruvic transaminase*	*Alkaline phosphatase*	*Bilirubin*
Donors	40	—	26 ± 48	29 ± 74	2.6 ± 1.9	0.2 ± 0.2
1	15	½- 1½	191 ± 162	524 ± 367	15.9 ± 8.8	0.7 ± 1.1
2A	7	10-14½	634 ± 432	975 ± 444	19.2 ± 12.8	0.5 ± 0.3
2B	5	8- 9½	353 ± 220	174 ± 141	12.8 ± 3.6	1.2 ± 1.7
2B	5	11½-15½	940 ± 981	1788 ± 1330	12.4 ± 6.0	0.8 ± 0.6
2C	5	8- 9½	291 ± 208	568 ± 754	8.8 ± 2.6	0.7 ± 0.4
2C	3	24¾-25⅙	367 ± 29	472 ± 158	6.1 ± 2.5	0.5 ± 0.3

Mean values and standard deviation given.

served as an oxygenator for the recirculated fluid (Fig. 2) which passed through a glass-wool filter and a bubble trap. Two occlusive roller pumps were used outside of the chamber. The oxygen compression of 40 pounds per square inch gauge pressure was reached within 20 minutes after inserting the organs. Various subgroups of experiments differed in the following important details:

A, low flow, rapid decompression. — Seven of the homografts were preserved for 10 to 14-1/2 hours. The portal venous and hepatic arterial flows were low, approximately 2.4 milliliters and .6 milliliter per gram per hour, respectively. Before removal of the homografts from the chamber, hyperbaric decompression was carried out for an interval of 25 to 35 minutes.

B, low flow, slow decompression. — The perfusion rate was the same as that used in A but the decompression time for the chamber was slower, having been extended to 3 to 4 hours. Five homografts were stored for 8 to 9-1/2 hours, and 5 more for a period of 11-1/2 to 15-1/2 hours.

C, higher flow, slow decompression. — The flow rate to the 2 inflowing vessels was doubled, approximately to 4.8 and 1.2 milliliters per gram liver per hour for the portal vein and hepatic artery, respectively. Five homografts were transplanted after preservation for 8 to 9-1/2 hours. Five more were preserved for 24-3/4 to 25-1/6 hours.

Group 3, oxygenation at atmospheric pressure and perfusion with diluted blood. Three homografts were preserved for 20-1/4 to 25-1/4 hours under the same conditions as in group 2C, except that 100 per cent oxygen was used at atmospheric pressure.

Group 4, hyperbaric oxygenation and perfusion with noncellular fluid. The conditions were the same as in group 2C, except that blood was not added to the perfusate. Storage for the 5 homografts ranged from 21-1/4 to 24-1/2 hours.

Group 5, hyperbaric oxygenation. Three homografts were preserved for 22-1/2 to 25 hours under the same conditions as in groups 2B and 2C, except that continuous perfusion was not provided. In 2 instances, the cooled grafts were immersed in a basin of noncellular perfusion fluid within the refrigerated chamber. In the other, perfusion was carried out with the same solution for 30 minutes at 6 milliliters per gram tissue per hour to ensure core cooling. The pumps were then turned off. The organ was not immersed in fluid.

RESULTS

Group 1. Two of the control dogs died of hepatic artery occlusion after 4 and 7 days, and 2 died of rejection in 7 and 8 days. The 8 remaining controls lived for 3 weeks or more, 6 surviving for at least 6 weeks. Using a maximum credit of 70 days for any individual dog, the mean survival time for the group was 38.7 ± 29.8 days, S.D. These results indicated that the immunosuppression being used was effective.

There was evidence of moderate ischemic injury as judged by liver function tests obtained in the recipient 18 to 24 hours postoperatively (Table I), but these changes were highly reversible. There were no unusual problems with operative hemorrhage. On the day of transplantation, the dogs received 400 to 500 milliliters of blood as well as 500 to 1,000 milliliters electrolyte solution.

At autopsy, the liver homografts were similar to those from previously reported series (16). In the hepatic homografts from the 4 dogs that died at 7 to 21 days after rejection, there was dense infiltration by large lymphoid cells and plasma cells around the portal and central veins. There was also necrosis of the majority of the hepatocytes in the central and middle zones of the liver lobule together with collapse and condensation of the reticulin framework of the central parts of the lobules. The hepatic homografts from 2 of those that survived longer were normal. In the other 2 grafts, there were bands of condensed reticulin linking adjacent central veins, slight lymphoid cell infiltration in the central and portal areas, and plugs of bile pigment in the centrilobular bile canaliculi.

Group 2. In the subgoup A, large numbers of gas bubbles appeared in the perfusion lines during rapid decompression. Most of the homografts had a spongy consistency due to similar gas emboli within the parenchyma, an observation which was confirmed roentgenographically. The organs generally gained weight, the average increase during residence in the chamber being 16.6 per cent.

After revascularization, the livers did not develop outflow block or regional infarcts, and after 30 to 60 minutes, they appeared to be perfused relatively homogeneously. At, or just before, this time, a hemorrhagic diathesis was evident to some degree in every dog despite prior therapy with epsilon amino-caproic acid and protamine sulfate. On the day of operation, at least 1,000 milliliters of blood and 2,000 to 3,000 milliliters of electrolyte solution were required. When insufficient quantities of the latter fluids were not provided, there was a drastic hemoconcentration with hematocrits as high as 76 per cent.

Six of the 7 dogs survived operation; the seventh died within a few hours for reasons not apparent at autopsy. The remaining dogs lived for 2, 3, 5, 5, 7 and 9 days, but their courses were not satisfactory. They were slow to recover from anesthesia, in some instances requiring several days to awaken fully. Two had generalized convulsions within minutes after homograft revascularization, a complication which was suspected to be caused by air emboli originating from the grafts. None were ever able to eat. In several dogs ascites and anasarca rapidly developed. Four of the 6 dogs which survived operation eventually had perforations of the duodenum, esophagus, or gallbladder.

On the day after homotransplantation, there was biochemical evidence of severe ischemic injury (Table I) which could be detected in the results of all the enzyme determinations. Subsequently, these values tended to return toward normal. An elevation in bilirubin to more than 1 milligram per hundred milliliters was seen in only 1 dog during the first 3 postperative days. However, there was severe and persistent depression of serum proteins to as low a 3.5 grams per hundred milliliters.

Histologic examination of the homografts showed evidence of rejection in those dogs that survived for 5 days or more, but there were no other unusual lesions. There was ischemic necrosis of the centrilobular hepatocytes in the homograft from the dog that died 3 days after transplantation. Another homograft, examined 2 days after grafting, appeared normal.

In subgroup B, those with low flow and slow decompression, the gross evidence of gas emboli was greatly reduced. The weight of the organs was stable during preservation, the average change being -0.6 per cent. Serious outflow block after transplantation was not observed.

The difficulties of supportive care for these dogs was less than those of group 2A, as reflected by a somewhat reduced requirement for blood and electrolyte solution on the day of operation. Arousal from anesthesia was more prompt.

Four of the 5 recipients of homografts which had been preserved for 8 to 9-1/2 hours survived operation, but 3 of these died after 4, 11, and 14 days. One dog is still alive after more than 4 months. With preservation for 11-1/2 to 15-1/2 hours, the results were similar. All 5 dogs lived through the operation, but 4 died later after 5, 6, 6, and 13 days. The other is still in good health after 5-1/2 months (Fig. 3).

The reason for the paucity of chronic survivors in this group was not

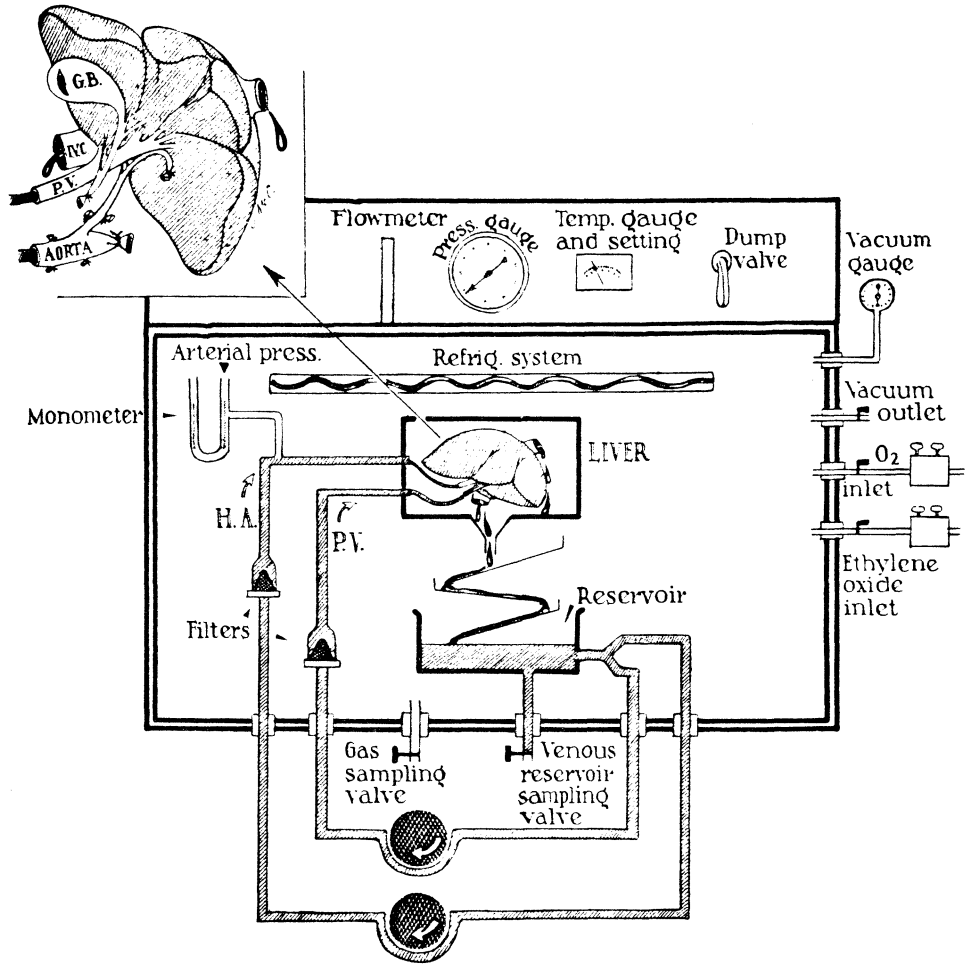

Fig. 2.— Preservation unit. The perfusion pumps are located outside of the hyperbaric chamber. The organ receptacle, the oxygenator, and the venous reservoir are inside. The various chamber inlets permit sampling of the perfusate, gas sterilization, and oxygen delivery and removal. The temperature is electronically controlled.

initially clear. There was biochemical evidence of acute ischemic injury, particularly in the organs which had been preserved for 11-1/2 to 15-1/2 hours (Table I). However, the postoperative changes after storage of 8 to 9-1/2 hours were not significantly different than in the controls of group 1 (Table I) and these returned toward normal with approximately equal rapidity. The rapid resolution of the enzyme changes did not imply a comparably fast restoration of total liver function. There was a severe depression of total serum proteins which had not returned to normal by the fifth postoperative day (Fig. 4).

Only 2 of the 8 homografts from experiments in which the recipients died in 2 weeks or less showed evidence of acute rejection. Evidence of healing ischemic centrilobular hepatocyte injury was present in the other 6 livers in this group. This was accompanied in 2 instances by centrilobular cholestasis. The degree of ischemic injury was not detectably different when preservation was for 8 to 9-1/2 hours as compared to 11-1/2 to 15-1/2 hours.

In group 2C, those with higher flow and slow decompression, the homografts preserved for 8 to 9-1/2 hours lost an average of 4.2 per cent of their weight during residence in the chamber, and seemed grossly to be in excellent condition. After subsequent revascularization, none developed outflow block. The 5 dogs which received these livers survived operation. They required an average of 700 milliliters of blood and 1,500 milliliters of electrolyte solution on the day of transplantation. Arousal from anesthesia was prompt.

Two dogs died after 9 and 19 days from rejection and multple liver abscesses, respectively. The other 3 lived for 34, 46, and 97 days. With a maximum credit for individual dogs of 70 days, the mean survival was 35.6 ± 23.9 days S. D.

The degree of ischemic injury which was detectable with liver function studies on the first postoperative morning was comparable to that in the control experiments (Table I). Moreover, the depression in serum protein was not so great as in dogs of groups 2A or 2B, and it had returned to normal within 5 days (Fig. 4).

The homografts preserved for more than 24 hours under the same conditions were less satisfactory. During storage, they gained an average of 4.3 per cent weight. After transplantation, they appeared to be well perfused, but an uncontrollable hemorrhagic diathesis developed in 2 of the recipients. In the other 3, liver function tests on the next morning were more abnormal than had been the case with the shorter interval. Nevertheless, only 1 of these 3 dogs died early, after 8 days; another died after 46 days. The third dog is still alive after 4 months (Fig. 5).

Pathologic studies of the livers preserved for the shorter time showed that 2 were undergoing rejection after 9 and 34 days. In both, dense infiltrations of lymphoid cells were present around the central and portal veins and there was marked necrosis of the central and midzonal hepatocytes. Two other hepatic homografts, from dogs which survived 19 and 46 days, respectively, showed some residual centrilobular scarring with atrophy of the hepatocytes immediately adjacent to central veins and

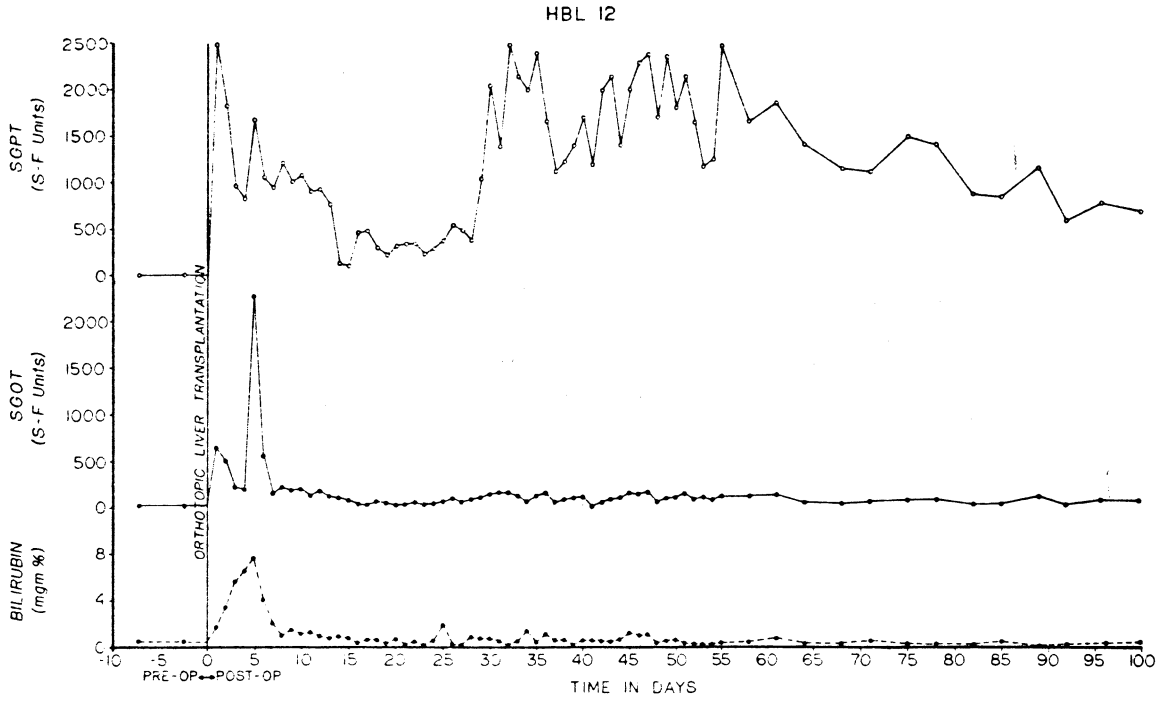

Fig. 3.— The first 100 postoperative days of a dog which received an orthotopic liver homograft after preservation for 14-1/2 hours. The total liver perfusion was only 3 millimeters per gram tissue per hour, a rate thought to be inadequate. Note the early evidence of reversible ischemic injury. The later rise in serum glutamic pyruvic transaminase was probably due to low grade rejection. The dog is still alive after 5-1/2 months. This dog, as well as all others in the study, was treated with azathioprine, a short postoperative course of prednisone, and antilymphocyte globulin.

increased numbers of reticulin fibers around the vein walls. Centrilobular cholestasis was also a feature of both livers. The 2 livers which were preserved for more than 1 day and recovered within a few hours after revascularization showed necrosis of the centrizonal hepatocytes but no other injury. The other, which was studied 8 days later, showed extensive necrosis, only those hepatocytes adjacent to the portal tracts surviving. There was no evidence of rejection.

Group 3. The oxygen pressure of the perfusate was maintained at 250 millimeters of mercury or more. The weight gain of the 3 homografts averaged 27.1 per cent during preservation. The livers had a patchy discoloration. All 3 gallbladders had grossly necrotic foci in the mucosa, and in 2 of these extensive intramural hematomas developed during the extracorporeal perfusion. After transplantation, severe outflow block was immediately evident with consequent extreme portal hypertension. The 3 recipients of these badly injured organs all died within 6 to 28 hours of acute hepatic failure and intra-abdominal hemorrhage.

Histologic examination of the 3 livers showed widespread necrosis of hepatocytes, only a few surviving immediately beneath the capsule.

Group 4. The homografts which lost an average of 1.9 per cent weight appeared grossly to be well preserved. There were no mucosal defects in the gallbladder. After transplantation, none of the livers developed outflow block. Nevertheless, 4 recipients of these organs died of acute liver failure and intra-abdominal hemorrhage within a few hours after operation. The fifth, which received a homograft that had been stored for 21-1/4 hours, had a serum glutamic pyruvic transaminase of 1,600 on the first postoperative day but lived for 9 more days with poor liver function throughout this particular period.

Only 1 of the homografts from the dogs which died early showed extensive necrosis of hepatocytes. In the other 3 livers, the hepatocytes in the central zones of the lobules were dead, but the others looked viable. There were no other changes. The homograft studied after 10 days showed evidence of rejection. The portal and central areas of the lobules were densely infiltrated by mononuclear cells, many of which possessed pyroninophilic cytoplasm. There was also hemorrhagic necrosis of the central and midzonal hepatocytes.

Group 5. The average weight loss of the homograft was 4 per cent. Slough of the gallbladder mucosa was severe in 1 of the homografts and minor in the others. Otherwise, the livers seemed to be in good condition.

After transplantation, 2 of the recipients died within 12 to 18 hours of acute liver failure. The other dog, which had a serum glutamic pyruvic transaminase of 2,000 on the first postoperative day, never fully awakened from anesthesia and died after 3-1/2 days.

The homografts from dogs which died within 18 hours showed widespread necrosis of liver cells afftecting all parts of the lobules. After 3-1/2 days, the other organ showed centrilobular hepatocyte cholestasis. There were also bile casts in the larger bile ducts.

DISCUSSION

A quantitative biochemical analysis of the response of canine liver tissue to ischemia has been published by Sicular and Moore. The ability of liver slices to oxidize glucose normally was retained for 30 to 60 minutes after death under normothermic conditions and for several hours if the tissue was refrigerated. These and other studies, such as those by Van Wyk and his associates, provided ample proof that the injury which follows cessation of hepatic circulation is a graded one which can be considerably slowed by the important emergency measure of immediately cooling the organ.

Unfortunately, the provision of metabolically active hepatic tissue does not necessarily mean that the organ can sustain life, particularly when the recipient animal is made totally dependent upon the homograft as is evident with orthotopic homotransplantation. This is well illustrated by the results reported herein. In some experiments, the quality of immediate liver function was sufficient to allow prompt recovery from anesthesia with subsequent long term survival. At the other end of the spectrum were dogs which died of liver failure within just a few hours.

Between these extremes were many examples of dogs with more or less grave acute hepatic insufficiency. The operative and postoperative

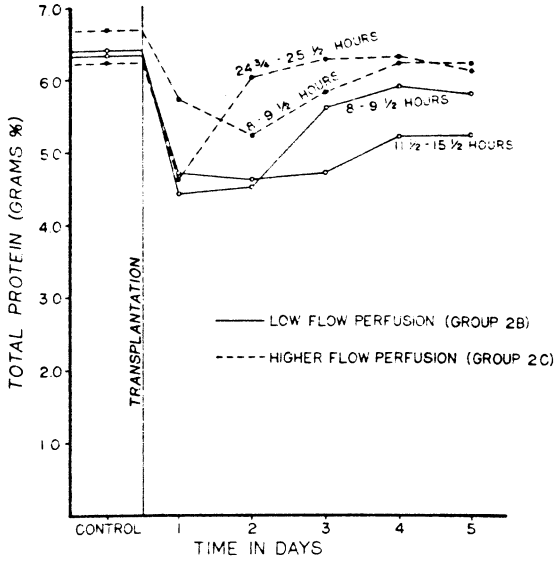

Fig. 4.— The effect of both preservation time and perfusion rate upon circulating plasma proteins. There is profound and sustained hypoproteinemia in the dogs which received grafts that had been preserved with low flow perfusion as compared with the results using a higher flow rate.

care of these dogs was difficult. A severe bleeding diathesis was invariable, which usually required multiple blood transfusions, extraordinary mechanical efforts at hemostasis, and the administration of clot-promoting agents, such as epsilon aminocaproic acid and protamine sulfate. The clotting defect, which has been shown by von Kaulla and his associates to be partly due to increased levels of circulatory fibrinolysins, is a complex one which is currently under study.

In such instances, control of bleeding was only the first step. Intensive therapy was required with electrolyte solutions or plasma, apparently because of the rapid formation of a third fluid space particularly within the abdomen but also in peripheral tissues. Detoxification of the barbiturate anesthesia was slow. The rapid fall of serum protein levels was an ominous sign. There was a high incidence of acute gastrointestinal ulcerations. With intensive care, these dogs could often be kept alive for several days, but they would not eat. Almost all died in less than 10 days. Although the homografts which were examined at autopsy often had evidence of early rejection in spite of immunosuppressive therapy, the predominant histologic changes were those of healing ischemic damage.

In this study, a number of modifications of preservation technique were tried, the condition of hypothermia being identical in each instance. The most successful method was with an adaptation of the system described for kidneys by Ackermann and Barnard, using a combination of hypothermia, hyperbaric oxygenation, and divided perfusion with diluted blood through the portal vein and hepatic artery at a total flow rate of 6 milliliters per gram of liver per hour. Both immediately and later, homografts which were stored under these conditions for 8 hours performed approximately as well as livers transplanted without delay. When attempts were made to extend the preservation time to 24 hours, only 3 of 5 recipients survived operation, although 2 of these lived for at least 6-1/2 postoperative weeks.

Systematic variation from the protocol already mentioned were evaluated to determine which details of technique were important and which were nonessential. Every deviation tried led to a deterioration of the results. Thus, a reduction in the rate of perfusion by half resulted in a demonstrably greater ischemic injury during storage for 8 hours. By using preservation for 24 hours as the test system, life-sustaining homografts could not be obtained if either hyperbaric oxygenation or perfusion, or even the blood fraction of the perfusate, were eliminated.

Beneficial effects of cold and perfusion for the preservation of organs are neither surprising nor difficult to explain. The reason for the apparently important role played by hyperbaric oxygenation is less clear. It is conceivable that the principal value of hyperbaria in these studies was that it increased the efficiency of the simple oxygenator which was kept within the compression chamber. Less likely is the possibility that oxygen diffusion through the surface of the organs was a significant factor, since this is thought to occur only to a depth of a few millimeters.

Manax and his associates have suggested other intriguing hypotheses. They have drawn attention to the metabolic inhibition which accompanies high atmospheric pressure, a desirable effect which has been

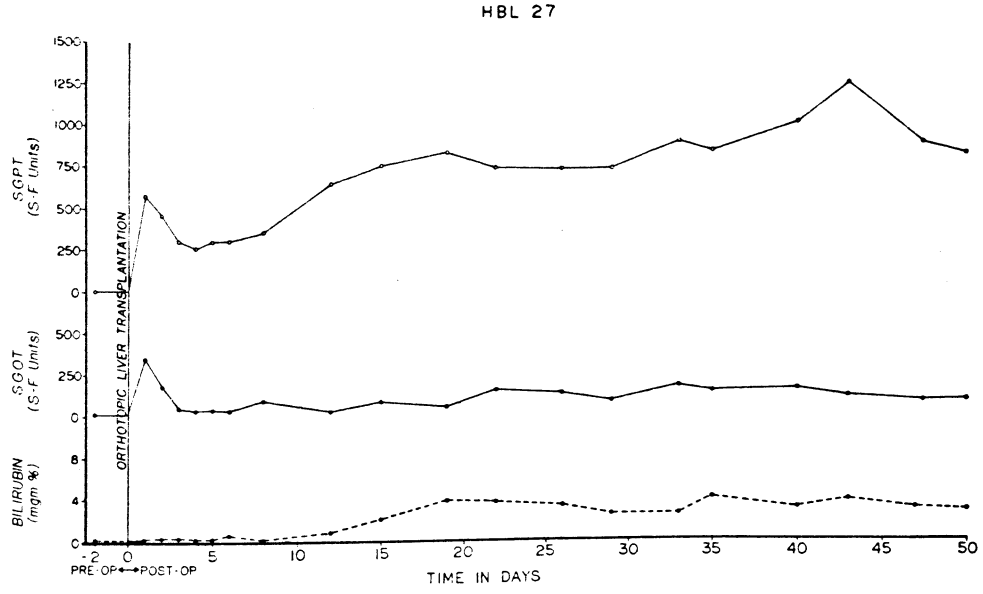

Fig. 5.— The course of a dog which received an orthotopic liver homograft which had been preserved for 24-3/4 hours. The perfusion rate was twice that used for the experiment shown in Figure 3. There is relatively minor biochemical evidence of early ischemic injury. The dog is still alive after 4 months.

demonstrated by Lyons and his colleagues to occur with nitrogen as well as with oxygen. Finally, mechanical influences, such as compression of the vascular system, may be helpful by preventing the formation of edema. It is of interest in our experiments that substantial weight gain of the preserved organs occurred only when hyperbaric oxygenation was omitted and that it was only in these homografts that outflow block was consistently observed.

The demonstration that livers can be kept in good condition for at least 8 and often for 24 hours after the death of the donor may have important clinical implications. It should be possible with this amount of time to prepare a patient to receive a homograft without the sense of urgency which has characterized past efforts at human liver transplantation. Preliminary analysis of donor and recipient histocompatibility antigens could be carried out in the interval.

However, other factors may diminish these hopes. In the laboratory, the timing of donor and recipient procedures can be precisely co-ordinated. The homograft which is taken from a cooled donor has a blood supply until virtually the moment of its excision. These ideal conditions are not attainable with cadaveric donors, since failing organ perfusion has invariably been present for sometime before death and because the liver must usually be in a normothermic postmortem state for some minutes before cooling can be instituted. At present, the extent to which the acceptable margin of ischemia has been dissipated before protective measures can be started appears to pose a more serious problem than that of efficient subsequent preservation.

SUMMARY

Orthotopic liver transplantation was performed with homografts that had been preserved for 8 to 25 hours after their removal from nonrelated mongrel donors. The fate of the recipients was compared to that of control dogs which received homografts transferred as rapidly as possible from cooled living donors. All dogs were treated postoperatively with immunosuppressive agents.

Homografts could be effectively preserved for 8 hours and often for as long as 24 hours with a combination of hypothermia at 4 degrees C., hyperbaric oxygenation at 3-1/2 to 4 atmospheres, and divided perfusion with diluted homologous blood through the portal vein and hepatic artery at a total rate of 6 milliliters per gram tissue per hour. At the end of the conservation period, the chamber was decompressed during 3 to 4 hours. The short term and long term course of 5 recipients of livers preserved for 8 hours was not different from that of control dogs. All dogs survived operation, and only 1 dog died in less than 19 days. Three of 5 recipients of livers preserved for 24 hours survived operation and 1 of these is still alive after a period of 4 months.

Deviations from this technique were evaluated with additional groups of 3 to 5 experiments in which livers were stored for 24 hours. Omission of perfusion or reduction of its rate by half, elimination of hyperbaric oxygenation, rapid chamber decompression, or failure to add homologous blood to the perfusate all resulted in less satisfactory results. The recipients of these homografts either died during operation or within a few hours or days thereafter. They usually had grave clotting abnormalities, evidence from serum enzyme measurements of severe hepatocellular injury, retardation of protein synthesis, and a high incidence of gastrointestinal ulceration. The impression that these livers had suffered from ischemic necrosis was confirmed by histologic studies.

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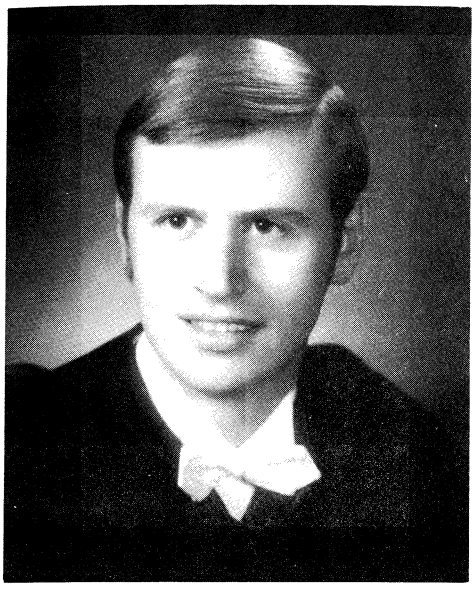
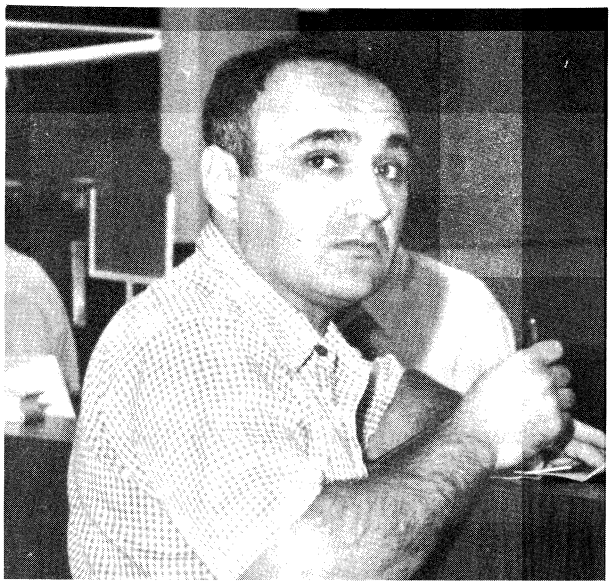


Dr. William J. Wall at the time of his tenure with Calne at Cambridge, England. Wall is now Chief of the Liver Transplantation Unit at the University of Western Ontario, London, Ontario, Canada.



Joseph Benichou was photographed at the airport on the way back from Denver to France in 1977.

In his studies, Benichou showed that the liver could tolerate much more cold ischemia induced with lactated Ringer's solution than previously realized and that a further increment in safe storage time could be obtained with Collin's or plasma-like solutions. Wall showed the same thing with the plasma-like solution brought to Cambridge from Holland where it had been developed by Schalm. These two articles marked the beginning of organ shipment from distant cities. Previously, the donor usually was brought to the recipient hospital, and the liver was prepared in an adjacent operating room.

Presently, Benichou is working at the University of Kremlin-Bicétre, Paris, France, and Wall is Chief of Liver Transplantation at the University of Western Ontario, London, Ontario, Canada.

Canine and human liver preservation for 6 to 18 hr by cold infusion

Transplantation, 24: 407-11, 1977

Joseph Benichou, Charles G. Halgrimson, Richard Weil, III, Lawrence J. Koep and Thomas E. Starzl

SUMMARY

Forty-one dog livers were preserved with cold, lactated Ringer's, plasma, or intracellular (Collins) solutions. Consistent survival was obtained with all three solutions for 9 hr. After 18 hr, the plasma and Collins solutions permitted survival, with the Collins solution having a slight overall advantage. The method using Collins solution has been used to preserve seven human livers in Los Angeles, to transport the organs to Denver, and to transport them as orthotopic grafts from 6 hr, 45 min to 10 hr later.

It has been well known for a decade that the preservation of whole animal and human livers is possible for many hr, as proved by subsequent life-sustaining function of the hepatic grafts in recipients (1-4, 7, 8, 10). The preservation methods can be divided into the more complicated ones involving continuous or intermittent perfusion (1-4), and those using simple intravascular infusion of cold solutions (7, 8, 11, 13) followed by cold storage.

In this investigation, infusion solutions have been compared in cold storage experiments to determine the extent, if any, that the infusion fluid composition affects the outcome. The results have been used to design a successful clinical trial of liver graft preservation and transport over long distances.

MATERIALS AND METHODS

Canine studies. With preservation times of 9 to 18 hr, three solution types were compared. After removal, the livers were washed free of blood with 500 to 800 ml of lactated Ringers' solution at 4 C. The fluid was delivered through the portal vein at 100 cm H2O pressure. This was followed with 400 to 600 ml of the test solution at 4 C. After placing it in a plastic bag, the organ had more test solution poured over it. The plastic bag was kept cold by packing it in ice, as is standard practice with kidney preservation (5, 6).

There were 41 experiments that were divided into 7 groups depending upon the solutions used and the time of preservation (Table 1). As the graft vessels were being anastomosed to the recipient structures, the preservation fluid was washed out with a slow final infusion of cold, lactated Ringer's solution. The transplantation technique was the one originally described in our laboratories (11), as slightly modified later (10). No immunosuppression was given postoperatively.

Human studies. The seven livers were removed in six different Los Angeles hospitals under conditions of heart-beating brain death. Preservation was with Collins solution, using the same technique as described in dogs but with adjustments of infusing volume according to the size of the organ. The donors were 10 months to 43 years old. These livers were

TABLE 1. Canine experimental groups

Group	No. of dogs	Preservation time (hr)	Infusion fluid
A	8	1	Lactated Ringer's
B_1	6	9	Collins I[a]
C_1	6	9	Plasma solution II[b]
D_1	3	9	Lactated Ringer's
B_2	7	18	Collins
C_2	8	18	Plasma solution
D_2	3	18	Lactated Ringer's

[a] Commercially available solution (code 5A 7810) which, after the recommended additives, contains for 1 liter: potassium, 115 mEq; sulfate, 60 mEq; magnesium, 60 mEq; chloride, 15 mEq; sodium, 10 mEq; bicarconate, 10 mEq; and phosphate, 100 mEq.

[b] Shalm's solution (*8*) as modified by Calne et al. (*4*) contains: dog plasma, 1 liter; heparin, 2,000 IU; hydrocortisone, 250 mg; ampicillin, 500 mg; dextrose, 250 mg; magnesium sulfate, 5 ml of a 10% solution; and potassium phosphate, 15 mEq.

TABLE 2. Data of the 7 human liver transplantations with preservation procedure

OT no.	Age of		Recipient diagnosis	Total ischemia	Solution amounts (ml)	
	Donor	Recipient			Lactated Ringer's	Collins
OT113	22 months	3 years	Biliary atresia	6 hr, 45 min	500	400
OT 116	4 years	7 years	Neonatal hepatitis	7 hr, 30 min	600	500
OT 118	43 years	24 years	α_1-antitrypsin deficiency	8 hr, 52 min	1300	800
OT 125	10 months	10 months	Biliary atresia	6 hr, 34 min	600	400
OT 126	7 years	11 years	Biliary atresia	7 hr, 20 min	1000	650
OT 127	3½ years	5½ years	α_1-antitrypsin deficiency	9 hr, 30 min	1000	600
OT 128	17 months	3½ years	Biliary atresia	10 hr	1000	500

initially flushed with 500 to 1,300 ml of lactated Ringer's solution and subsequently with 400 to 800 ml of Collins solution (Table 2). The final flushing of the organ with lactated Ringer's solution during implantation was the same as that with the dog. Transplantation was by previously reported techniques (10-12).

RESULTS

Animal Studies

There was minimal acute ischemic damage of the grafts in control animals that had immediate transplantation of cooled livers (Fig. 1). Rejection supervened within a few days. Five of the control animals lived for at least 7 days.

9 hr. With preservation for 9 hr, the survival and behavior of the animals were similar to those of the controls. Furthermore, there were no differences by the criteria of postoperative behavior and mortality with lactated Ringer's, Collins, or plasma solutions. Nevertheless, biochemical evidence of ischemic damage was less with Collins solution than with lactated Ringer's or plasma solutions (Fig. 1).

One animal survived with no immunosuppression and is alive 3 months later.

18 hr. Recipients of three livers preserved with lactated Ringer's solution died within 1 day. With plasma and Collins solution, about one-half of the recipients survived the immediate postoperative period, in spite of the fact that moderate to severe ischemic injury was always present (Fig. 2). In terms of both survival and liver function, there seemed to be a slight advantage with Collins solution compared with the plasma (Fig. 2).

Human Studies

Six of the seven cadaveric livers functioned well after 6 hr, 45 min to 10 hr of preservation, with prompt relief of jaundice and generation of good clotting factors. The serum glutamic-oxaloacetic transaminase always rose (Fig. 3), but the increase was to above 1,000 in only two of the six

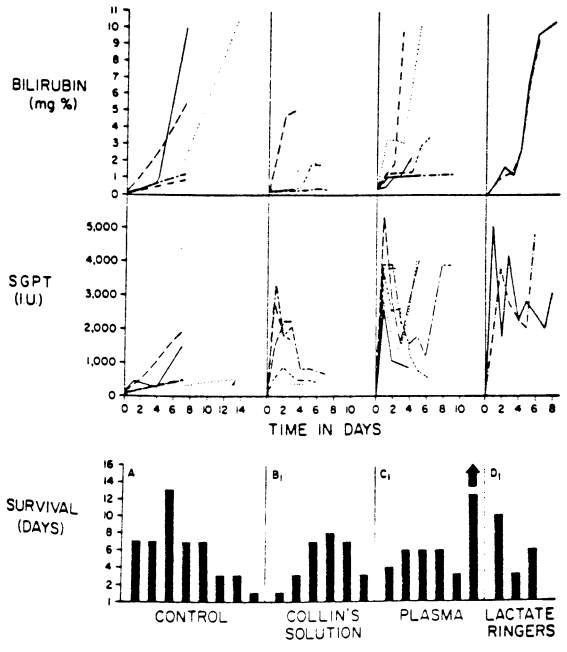


Fig. 1.— The effect of preservation for 9 hr upon orthotopic liver grafts in dogs. In the control experiments, transplantation was carried out immediately after flushing with cold, lactated Ringer's solution and after preservation for 1 hr or less. Although immunosuppression was not used, one animal in group C1 (arrow) is still alive after 80 days.

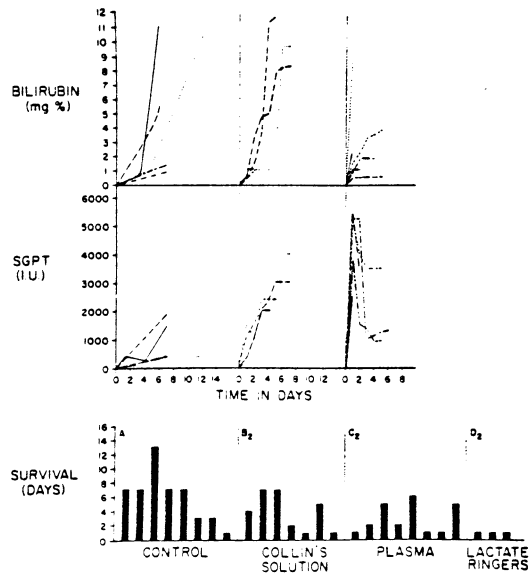


Fig. 2.— The effect of preservation for 18 hr upon orthotopic liver grafts in dogs. The experimental and control conditions were the same as for Figure 1.

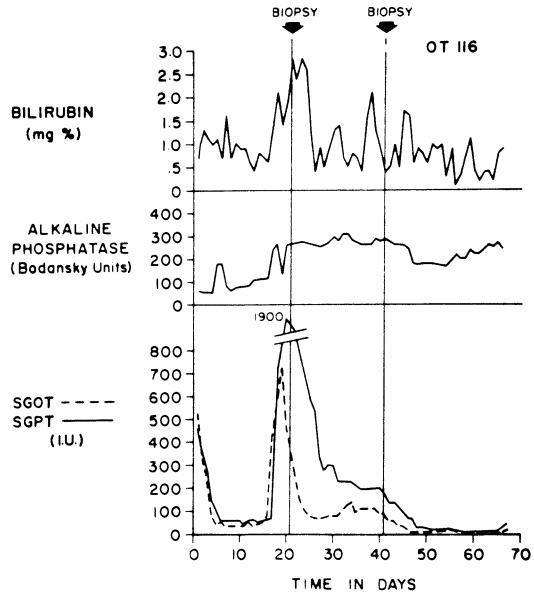


Fig. 3.— The course of a 7-year-old child who received a liver that was removed in Los Angeles, preserved for 7-1/2 hr, flown 1,000 miles to Denver by a commercial airliner, and transplanted. Initial function was excellent. A biopsy taken 2-1/2 weeks later, when homograft function deteriorated, was interpreted as hepatitis. The recipient is well 6 months postoperatively.

patients, and in these recipients there was a return to normal within 3 to 10 days. Five of these six patients are still alive from 3 weeks to 8 months post-transplant. The patient who died had normal liver function but developed lethal cardiopulmonary complications.

The seventh patient was 30 years old. At operation, the liver could not be completely revascularized because of thrombosis of the recipient's portal vein. The graft functioned poorly, and there was an increase of serum glutamic-oxaloacetic transaminase to more than 3,000 IU (normal <50).

Eleven days later, another donor became available. Retransplantation was performed but the second homograft that was transplanted with the usual cooling (10,11), without prolonged preservation, behaved the same as the first and the patient died 2 days later. The histopathological changes were much the same in the first and second organs, including wide spread ischemic changes.

DISCUSSION

The intravascular infusion of cold electrolyte solution was the first effective technique of liver preservation (11), and it was not surprising that the same general method immediately became the standard for the quick cooling of kidney (9) and other organs.

However, in the early experience with canine liver transplantation, it was found that delay of more than 2 hr after this kind of cooling was not compatible with survival. From our present results, it is obvious that the time of 2 hr was a gross underestimate of what can be expected after simple infusion, providing other aspects of the operation and care are of high caliber. Even after 9 hr the results with lactated Ringer's solution alone were almost equal to those achieved with the complex solutions. Thus, the most important ingredient of success, no matter what the solution, was organ hypothermia.

In our experiments, the extra value of special solutions became clear only with longer preservation times. At 18 hr, both the plasma solution of Shalm et al. (8) modified by Wall et al. (13) and the Collins solution yielded better results than lactated Ringer's . Lambotte et al. (7) have previously shown the supplementary value of Collins solution for liver preservation.

If Collins solution is used, or for that matter the plasma solution preferred by Calne et al. (3), the safe time for liver preservation is extended to at least 9 hr and probably considerably beyond this. With this much time, the shipping of livers between most major American cities has become a real possibility. The implications of such a development were emphasized by Calne et al. (3) in their efforts at liver procurement in Europe.

The precaution of final flushing with lactated Ringer's solution should be scrupulously taken if the Collins solution is used. In one of our patients, this step was omitted. When the organ was revascularized, the serum potassium abruptly rose to 6.5 mEq/p 1,000 ml, followed by cardiac arrest from which resuscitation fortunately was possible. The sudden efflux of the potassium-rich intrahepatic fluid with revascularization of the unflushed graft was undoubtedly responsible.

Acknowledgments. The organ procurement was organized and carried out by Shawney Fine, Natalie Nankin, Barbara Schulman, and Roger Smith, coordinators of the Terasaki Organ Procurement Program. Paul D. Taylor, Senior Instructor in Surgery, at the University of Colorado Medical Center arranged and carried out the organ transfer to Denver, Colorado.

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Simple hypothermic preservation for transporting human livers long distances for transplantation
Report of 12 cases

Transplantation, 23: 210-16, 1977

W. J. Wall, R. Y. Calne, B. M. Herbertson, P. G. Baker, D. P. Smith, J. Underwood, A. Kostakis and R. Williams

SUMMARY

Since 1973, 12 patients in the Cambridge-King's College Hospital liver transplant programme have received livers from donors dying in hospitals considerable distances from the transplant centre in Cambridge. The method of preservation used to transport these livers from 35 to 110 miles was flush perfusion with plasma protein fraction and hypothermic storage in ice. The ischaemia times ranged from 2 hr and 42 min and to 4 hr and 22 min. All of the recipients had good or excellent postoperative function of the grafts and 6 of the 12 recipients are alive, the longest survival being 29 months. The preservation technique is simple and easily portable and has given reliable 4-hr preservation of the human liver.

There is a shortage of donor organs in the United Kingdom (5). Suitable candidates for transplantation with fatal liver disease often cannot wait weeks or longer for a donor to become available in their own hospital. We have moved the sick recipient and the transplant team to the donor institution for the operation but this is not a practical procedure. These circumstances have made it necessary to remove livers from donors dying in distant hospitals and transport them to Cambridge for transplantation. This poses special problems for liver grafting because of the extreme sensitivity of this organ to ischemia and the difficulty in preserving the human liver for extended periods using simple, readily portable methods.

In 1969 two patients in the Cambridge-King's College Hospital series underwent orthotopic liver transplantation using donor livers that had been transported 68 and 119 miles and were ischaemic for 4-3/4 and 5-1/4 hr, respectively. One recipient (OL-15) died from massive hepatic necrosis 7 days after operation; hepatic insufficiency contributed to the death of the other patient (OL-13). Although many factors were involved in these two cases, poor liver preservation for these relatively long ischaemia times was a major factor in the poor function of the grafted organs. The preservation method used was a modification of the technique described by Schalm (21). After flush perfusion with chilled heparinized Hartmann's solution followed by a plasma-bicarbonate-dextrose solution, the liver was stored in ice. This method was used successfully in the Cambridge-King's College Hospital liver transplant programme when both donor and recipient were in the same hospital and preservation times usually ranged from 1-1/2 to 3 hr (7,8).

In order to extend the safe period of liver preservation beyond 3 hr, investigations in pig liver preservation were carried out in Cambridge (9,10,12,17). Modifications in volume, additives, and pH of the preservation solution resulted in a perfusate that gave 12 to 17 hr of porcine liver preservation, and it was adopted for clinical use. The purpose of this paper is to report the subsequent 12 cases in the Cambridge-King's College Hospital series using simple hypothermic preservation in transporting human livers between hospitals for transplantation.

MATERIALS AND METHODS

Since 1973 12 human livers have been removed at distant hospitals and brought by car, plane, or helicopter to Cambridge for transplantation. The ischaemia times, distances travelled, and post-transplant function are shown in Table 1. One transplant team (surgeon, assistant, and one or two technicians) travels to the donor hospital and starts the donor operation. When it has been determined (after laparotomy) that the liver is suitable, word is sent to the recipient hospital where a second surgeon than begins the recipient hepatectomy. An attempt is made to synchronize the two procedures to allow the final steps in the removal of the recipient liver to coincide with the arrival of the donor organ.

Details of the donor operation have been described in detail elsewhere (6). In essence, after circulatory arrest of the donor, the operation is started. The portal vein is cannulated via the superior mesenteric vein and gravity infusion at 1 m of Hartmann's solution at 4 C containing heparin (2,000 IU/litre) is begun. The liver should immediately become cold, assuming a pale brown colour. The steps in the donor hepatectomy proceed after cooling has commenced. After infusion of 1,500 to 2,000 ml of

TABLE 1. Data on 12 patients who received orthotopic liver transplants from donors dying in distant hospitals

Case	Age (years)	Date of transplant	Diagnosis	Donor liver				Survival	Cause of death
				Warm anoxia* (min)	Ischaemia	Distance transported (miles)	Function		
1. OL-33	43	11/11/73	Cholangiocarcinoma	37	3 hr 56 min	54	Good	5 months	Metastases
2. OL-34	33	2/19/74	Hepatoma	14	4 hr 20 min	54	Good	29 months	Alive
3. OL-36	22	2/15/75	Cirrhosis + hepatoma	19	4 hr 22 min	50	Excellent	7½ months	Sepsis + metastases
4. OL-38	33	4/14/75	Cirrhosis	17	3 hr 57 min	62	Good	14 days	Pneumonia
5. OL-39	44	6/14/75	Cirrhosis	8	3 hr 53 min	90	Excellent	2½ months	Sepsis
6. OL-42	16	12/24/75	Hepatoma	2	3 hr 15 min	110	Excellent	7 months	Alive
7. OL-44	44	2/4/76	Hepatoma	0	2 hr 42 min	100	Excellent	5½ months	Alive
8. OL-45	35	2/12/76	Cirrhosis	7	3 hr 17 min	62	Good	5 months	Alive
9. OL-47	54	3/30/76	Hepatoma	0	3 hr 39 min	62	Excellent	1½ months	Sepsis
10. OL-48	48	4/12/76	Cholangiocarcinoma	0	3 hr 17 min	40	Excellent	3½ months	Alive
11. OL-50	44	5/5/76	Secondary carcinoma	0	2 hr 46 min	35	Good	5 weeks	Metastases and bowel obstruction
12. OL-51	56	6/16/76	Cirrhosis + hepatoma	0	3 hr 3 min	54	Excellent	5 weeks	Alive

* Time from ventilator switch off to commencement of liver cooling.

Hartmann's solution, the flushing solution is changed to ice-cold plasma protein fraction (PPF) containing additives (Table 2). Seven hundred ml of PPF are administered via the portal vein and a further 300 ml via the hepatic artery after removal of the donor liver. The liver is placed in a plastic bag containing physiological saline at 4 C and then into a second plastic bag. It is then surrounded by ice in an insulated container. The container itself measures 13 x 13-1/2 x 19 inches and is easily accommodated on a car seat. The liver is then transported with the transplant team to Cambridge by the quickest practical route.

It has been customary, in the United Kingdom in donors with brain death, to wait until the circulation has ceased before beginning operation to remove organs. With such donors it is not unusual for the heart beat to continue for 15 to 30 min after ventilator switch off. The warm anoxia suffered by the liver during this waiting period can be considerable. Although this practice was rigidly adhered to in the earlier cases, in the last few patients the operation has commenced immediately after ventilator switch off or ventilation has been continued until the liver has been skeletonized with an intact circulation (Table 1).

After recipient hepatectomy, the donor liver is grafted orthotopically anastomosing the suprahepatic cava, portal vein, infraheptic cava, hepatic artery, and biliary tract in that order. Postoperative liver function is measured by assessment of the clinical state of the recipient, daily liver function tests (serum bilirubin, albumin, aminotransferases, alkaline phosphatase, and prothrombin time), bile output from the T-tube (if one has been used), the presence or absence of a bleeding diathesis, and liver scans and needle biopsy when indicated. Liver function tests were classified as excellent if mild disturbances occurred immediately after transplant and rapidly returned to normal values in a patient who was clinically well. A satisfactory clinical state with moderately abnormal liver function tests in the first 2 to 3 weeks was classed as good. Unsatisfactory liver function leading or contributing to the death of the recipient in the postoperative period would have been classified as poor.

TABLE 2. Composition of preservation solution

To each litre of PPF is added:
2,000 IU of Heparin
250 mg of Hydrocortisone
500 mg of Ampicillin
6 ml of 0.1 N HCl
5 ml of 10% Magnesium sulphate
250 mg of dextrose
15 mEq of Potassium phosphate

Illustrative Cases

Case 2 (OL-34). This 33-year-old woman underwent liver transplantation for hepatoma on February 19, 1974. Preoperative liver functions were normal. The donor died of a head injury in a hospital 54 miles away. Fourteen min elapsed from the time of cessation of artificial ventilation until circulatory arrest when the donor liver cooling was started. Arterial blood gases at this point showed a pO_2 of 2, pCO_2 of 96, and pH 7.14. The liver was transported by car and was ischaemic for 4 hr 20 min. After transplantation she was generally well but had strikingly abnormal liver function tests as shown in Figure 1. After an immediate high rise and fall in SGOT and alkaline phosphatase, the serum bilirubin rose steadily to 30 mg/100 ml on the 12th day. Cholangiography demonstrated no obstruction in the biliary tree. Although the leukocyte migration inhibition test was negative, antirejection treatment with Solu-medrol was given at this point but the bilirubin fell only slowly over the next 3 weeks. The jaundice was considered to be because of ischaemic damage incurred during the agonal phase in the donor after ventilator switch off. We have, however, no direct evidence to substantiate this hypothesis. She was discharged 2 months after transplantation with normal liver function tests and is well 3 years later.

Case 3 (OL-36). This 22-year-old female underwent liver transplantation for hepatoma and cirrhosis on February 15, 1975. The donor liver was transported 50 miles by car and was ischaemic for 4 hr and 22 min. She was intensely jaundiced preoperatively with a bilirubin of 35.2 mg/100 ml. Post-transplant there was a dramatic drop in the bilirubin to 3.3 mg/100 ml by the 3rd day (Fig. 2). There was a transient rise in SGOT and SGPT which fell to normal by the 7th day. The patient survived 7 months and eventually died from sepsis and pulmonary and bone secondaries.

Case 5 (OL-39). This 44-year-old male underwent liver transplantation for cirrhosis on May 14, 1975. The donor died in a hospital 90 miles from Cambridge and the liver was ischaemic for 3 hr and 53 min. The recipient was well immediately postoperatively and did not become jaundiced until 6 weeks after transplantation when, at exploration, he was found to have a subhepatic abscess communicating with the biliary anastomosis. He died of a myocardial infarct and pulmonary oedema 2 days after the second operation.

Case 7 (OL-44). This 44-year-old male underwent liver transplantation for hepatoma on February 4, 1976. The donor died of a cerebral tumour 100 miles away and the liver was transported by plane to Cambridge. It was ischaemic for 2 hr and 42 min. His postoperative liver function was excellent and he had a smooth course in the hospital. He was discharged 4 weeks after operation and is well 5-1/2 months later.

RESULTS AND DISCUSSION

The post-transplant liver function and patient survival are summarized in Table 1. There were no deaths from liver failure in the postoperative period and all had good or excellent function of the graft. Five of 12

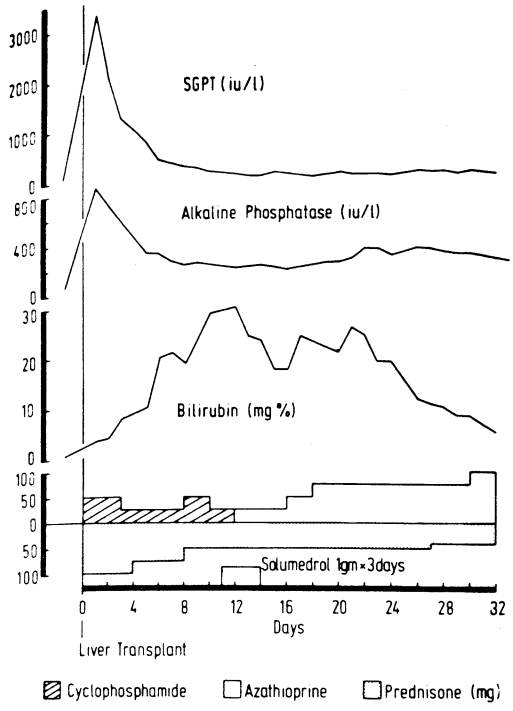

Fig. 1.— Liver function tests of case 2 (OL-34). After a quick rise and fall in SGPT and alkaline phosphatase, there was a continued rise in bilirubin over the first 12 days which only slowly fell over the ensuing 3 weeks. No specific cause for the jaundice was identified. The recipient is alive 3 years later.

livers were preserved for approximately 4 hr. One patient (case 4) died of pneumonia 14 days after the operation with good liver function. The histology of the liver at postmortem examination in this case is shown in Figure 3. The hepatocytes appeared to be healthy, with active liver cell regeneration in some areas.

Although continuous perfusion techniques have given extended successful liver preservation (2,4,22), they have the disadvantages of being expensive, technically complex, bulky, and not easily portable. Simple hyothermic preservation is desirable when one considers the logistics in moving livers over distances of up to 100 miles between hospitals. Although every effort is made to minimize the preservation time, transporting livers in this fashion may result in ischaemia times of 4 hr or more. The preservation method used in these cases has been very satisfactory for this length of time. During the last year the periods of both warm and cold ischaemia have diminished and despite transportation of livers from other institutions, ischaemia times have sometimes been short (Table 1).

The importance of the constituents of the preservation solution is undergoing investigation (13-16, 19, 20). The basic principle is to attempt to maintain the status quo of the cells during the period of preservation by the use of a solution that will prevent or ameliorate the potential electrolyte and water shifts that occur as cell membranes become incompetent at low temperatures. As the duration of the preservation time increases, the composition of the solution may become more important. The use of "intracellular" solutions has become popular, although the exact role of many of the anions, cations, and non-electrolytes is not yet clear. The use of the PPF in this study was the result of modifying the preservation solution used in porcine liver preservation described by Schalm (21). The heparinized chilled Hartmann's solution is used for initial rapid cooling of the organ. The flushing solution is then changed to PPF with additives which has an osmolarity of 330 to 340 mOsmol/litre. The PPF is prepared from whole stored blood by centrifugation and fractionation according to the method of Kistler and Nitschmann (18). This results in a solution in which the protein content is 92 to 96% albumin. It is essentially free of fibrinogen, Factor VIII, lipoproteins, and y-globulins. The additives listed in Table 2 are put in the solution just a few hr prior to use. Cortisone is added for its membrane stabilising effect. The addition of 6 ml of 0.1 N HCL results in a pH of approximately 6.8 This acid perfusate has given better experimental results than solutions that have a higher pH (3,11,23). Three liters of oxygen are bubbled through the solution shortly before use. The preservation fluids are stored in ice and taken with the surgical team to the donor hospital.

The role of the potassium phosphate and magnesium sulphate in the solution is not clearly defined, although good results have been obtained in renal (13-15,20) and liver (1,9,12) preservation with the use of solutions containing elevated levels of potassium and magnesium. Downes et al. (16) believe that prevention of cell swelling may be the mechanism of their action but reduction in changes in intracellular concentrations of these cations may be equally important.

Some potassium leaves the cells and acid metabolites accumulate in the liver graft during preservation and particularly during the rewarming phase (while the suprahepatic caval and portal vein anastomoses are being completed). To prevent the sudden entry of these substances into the recipient circulation the liver is flushed via the portal vein with 400 ml of PPF at room temperature (without additives) just prior to the completion of the portal vein anastomosis, whilst the inferior vena cava catheter below the liver is allowed to drain freely before it is clamped and the inferior vena cava catheter above the liver is declamped.

Without question the best test of liver function after hepatic transplantation is the ability of the grafted organ to sustain the life of the recipient. This was true in each of these 12 patients. An immediate rise in SGOT and SGPT after liver transplantation is a common finding as demonstrated in Figures 1 and 2. This probably reflects the ischaemic insult to the graft. The aminotransferases fall to normal levels within 1 week to 10 days if the preservation method has been satisfactory. Mild to moderate elevation of the serum bilirubin (both conjugated and unconjugated) during the first 2 weeks has also been a consistent occurrence in these cases. This results

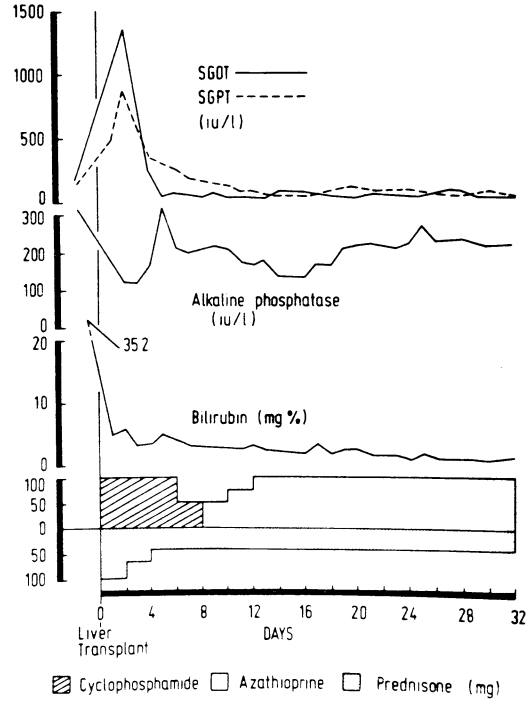

Fig. 2.— Liver function tests of case 3 (OL-36). There was a rapid fall in serum bilirubin after transplantation. The donor liver was ischaemic for 4 hr and 22 min. These liver function tests were classified as excellent.

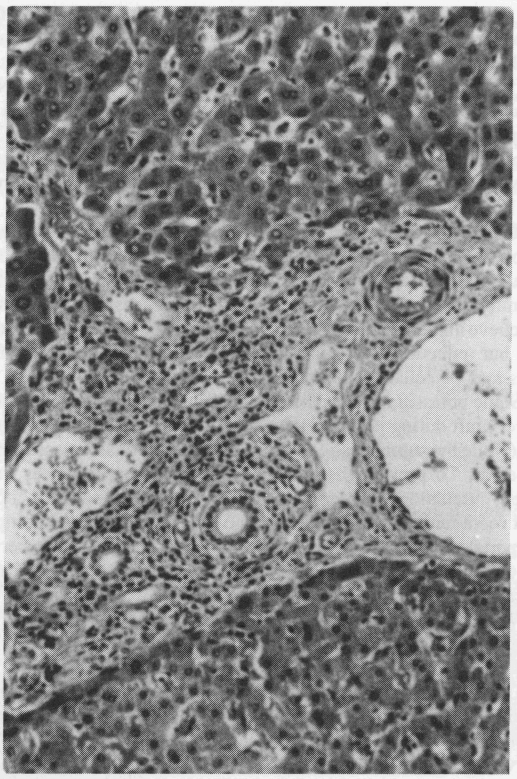

Fig. 3.— Liver transplant of case 4 (OL-3), postmortem specimen. A portal tract and peripheral parts of surrounding liver lobules are shown. Apart from a slight excess of mononuclear cells in the portal tracts the structure of the liver is essentially normal. There was a slight excess of liver cells in mitosis in other areas. Haematoxylin and eosin. X 145.

from hepatic parenchymal ischaemic damage and possibly some degree of intrahepatic cholestasis. Also, a considerable bilirubin load is put on the new liver from the large volumes of stored blood which are inevitably given during the operation.

Many other causes of abnormal liver function in the postoperation period (sepsis, cholangitis, early rejection, and drug toxicity) can be difficult to differentiate from liver injury resulting from the preservation technique or its duration. A major factor in the earlier cases in this study was the anoxic damage suffered by the liver cells whilst the surgeon anxiously waited for cessation of the circulation after ventilator switch off. Significant damage to the liver occurs under these circumstances and is manifest in the postoperative period by abnormal function, regardless of the excellence of the method of preservation. We believe this was the cause of the prolonged jaundice in case 2 (OL-34). Avoidance of this period in the last few cases has been reflected in less disturbance in liver function tests after transplantation.

A bleeding diathesis in the recipient after liver grafting is an ominous sign. None of the patients in this report had an abnormal bleeding tendency immediately after transplantation. The prothrombin time is measured frequently in the first 24 hr after grafting and a normal or near normal result is a sign of good liver function.

The preservation method described is simple, straightforward, and requires no elaborate equipment. It is easily portable and has given reliable 4-hr preservation of the human liver. This has allowed adequate time for the procurement of donor livers from hospitals considerable distances from the transplant centre. The waiting period for sick patients requiring liver transplantation has been shortened as a result.

Acknowledgements. The patients in this report are part of the combined Cambridge-King's College Hospital liver transplant programme. We are indebted to our medical and nursing colleagues at Addenbrooke's and King's College Hospitals for their help in the management of these patients. We thank the medical and nursing personnel of each of the hospitals where the donor livers were removed for their cooperation and assistance. The illustrations were kindly prepared by the Department of Medical Photography at Addenbrooke's Hospital.

Addendum. A further seven livers have been preserved by the above technique. All have had initial excellent function following transplantation. The longest preservation period was 7 hr 50 min.

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II

Part II
Immunosuppression

Part II
Immunosuppression

Immunosuppression is what gives organ transplantation specificity in comparison to all other surgical endeavors. The techniques of immunosuppression were developed in the laboratories and in humans with the simpler model of renal transplantation. For the most part, the latter were applied with little change to the transplantation of the liver, heart, heart/lung and pancreas.

The origin of the idea. Before Medawar, there was nothing. The possibility that there was an immune barrier to successful transplantation of tissues and organs apparently was not part of the consciousness of early clinicians, or for that matter of most basic scientists. This realization awaited the classical studies of Medawar with rabbit skin grafts.[1]

Appreciation by Medawar that rejection was an immunologic phenomenon made inevitable almost everything that followed. The deliberate depression of immunologic reactivity became feasible theoretically when total body irradiation[2, 3] and adrenal cortical steroids[4] were shown to be immunosuppressive. The next great step was the introduction of thiopurine compounds, 6-mercaptopurine and its imidazole derivative, azathioprine, which inhibited heterohemagglutinin formation in mice,[5] responsiveness to foreign proteins in rats[6] and rejection of skin and renal grafts in rats[7, 8] and dogs,[9, 10] respectively.

The human kidney transplant prototype. The kind of laboratory research just cited proved inapplicable to organ replacement in man. Complete control of rejection with a single agent rarely was achieved without lethal side effects either in animals or in man as exemplified by the historically important trials with total body irradiation[11] as well as by early trials with 6-mercaptopurine and azathioprine.[12-16]

Hopeful signs from the clinical experience through 1962 were footnotes to an otherwise dreary catalogue of failures. In 1961, Burnet, a Nobel laureate with Medawar the preceding year, wrote in the *New England Journal of Medicine:*

> Much thought has been given to ways by which tissues or organs not genetically and antigenetically identical with the patient might be made to survive and function in the alien environment. On the whole, the present outlook is highly unfavorable to success....[17]

The modern era of transplantation was entered when it was realized that azathioprine and prednisone had at least additive, and possible synergistic, effects.[18] Using living-related donors, renal transplantation became overnight a practical means of treating renal failure.[18, 19] Other multimodality techniques followed. (cf. Table I)

The most important new variable between 1962 and 1978 was the adjuvant use of antilymphocyte globulin (ALG) added to azathioprine (or to cyclophosphamide) and steroids.[20] Ultimately, it became possible to produce more potent and specific ALG's[21] with the hybridoma techniques discovered by Köhler and Milstein.[22] However, from 1963 to 1979 with any of the methods available, truly acceptable results were obtained only with renal transplantation from consanguineous donors. Candidates for liver transplantation were faced with the bleak prospect of receiving a non-related (cadaveric) graft.

The situation changed drastically for recipients of all kinds of cadaveric organs with the disclosure by Borel et al[23] of the phenomenal immunosuppressive qualities of cyclosporine; with the initial clinical trials of this agent for cadaveric renal transplantation by Calne et al;[24, 25] and with the systematic combination of cyclosporine with steroids and other immunosuppressive measures.[26, 27] (cf. Table I)

Application to liver transplantation. Liver transplantation at first was a passive partner in the development of immunosuppressive techniques. Whatever the current clinical practice or current laboratory focus in renal transplantation (cf. Table I), it was passed on for secondary consideration in liver transplantation and the transplantation of other extrarenal organs. In Denver, Colorado and in Cambridge, England, where both organs were at center stage, the delay between renal and hepatic trials with new drugs or practices almost disappeared.

TABLE 1: IMMUNOSUPPRESSIVE DRUG REGIMENS AND ADJUNCTS DEVELOPED WITH KIDNEY TRANSPLANTATION AND APPLIED FOR LIVERS

Agents	Year Described and Reported	Place	Deficiencies	Used for Livers
Azathioprine	1962 (12)	Boston	Ineffective, Dangerous	No
Azathioprine-Steroids	1963 (18)	Denver	Suboptimal	Yes
Thoracic Duct Drainage as Adjunct	1963 (28)*	Stockholm	Nuisance: requires 20 to 30 days pretreatment	Yes**
Thymectomy as Adjunct	1963 (29)	Denver	Unproven Value	Yes, Rarely in 1963
Splenectomy as Adjunct	1963 (29)	Denver	No Longer necessary	Yes, Once Commonly
ALG as Adjunct	1966 (20)	Denver	Suboptimal	Yes
Cyclophosphamide subsitute for azathioprine	1970 (30)	Denver	No advantage except for patients with azathioprine toxicity	Yes**
Total Lymphoid Irradiation	1979 (31,32)	Palo Alto, Minneapolis	Dangerous, extensive preparation, not quickly reversible	Yes***
Cyclosporine	1978-1979 (24, 25)	Cambridge	Suboptimal	Yes
Cyclosporine-Steroids	1980 (26,27)	Denver	Nephrotoxicity; limits dose: Rejection not always controlled	Yes

* It was not realized until much later that pretreatment for 3 to 4 weeks before transplantation was a necessary condition (33).

** These trials were summarized many years later with at least 10 years followup for surviving patients (34).

*** By Professor J. A. Myburgh of Johannesburg.

When cyclosporine eventually arrived on the scene, two of the first 34 patients in Calne's cyclosporine series were recipients of livers.[25] At the University of Colorado, only about 25 renal recipients were treated with cyclosporine in late 1979 and early 1980 before going forward on March 9, 1980 with the liver transplant trials that drastically changed the expectations regarding this operation.[27,34,35]

Although lessons learned with the kidney eventually proved to be applicable to liver transplantation, at the outset specific testing of immunosuppressive agents in canine liver recipients became a crucial issue. The reason was the great difficulty in the early 1960's in obtaining long-term survival of dog liver recipients under immunosuppression with total body irradiation[36] or azathioprine with or without steroids.[37,38] The concept that immune reactions damaging to the liver were organ-specific was particularly well stated by Moore et al.[38] The possibility existed that success was beyond hope of achievement.

Until this issue of feasibility could be resolved with absolute certainty in animals, the clinical trials of liver replacement that had begun in 1963[39] had to be stopped. Fortunately, truly long-term survival was obtained quickly in dogs,[40, 41] and one animal treated with azathioprine lived for almost 12 years.[42] However, it is worth noting that only four agents have been shown unequivocably to permit long-term survival after liver transplantation in dogs. These drugs are azathioprine,[40] antilymphocyte serum (ALS) or its globulin derivative (ALG),[20] cyclosporine or its analogue, Nva_2-cyclosporine[43, 44] and the new Japanese drug, FK506, that has qualities similar to cyclosporine.[45]

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This is the first known attempt at immunosuppression for experimental liver transplantation. The experiments were performed in 1959 and 1960 when irradiation still was envisioned to play an important role in immunosuppression for transplantation. All efforts failed. Treatment of the graft with high dose irradiation did not improve the results and treatment of the recipient seemingly reduced the chance of perioperative survival.

Canine liver homotransplants
The effect of host and graft irradiation

Archives of Surgery, 85: 460-4, 1962

T. E. Starzl, G. W. Butz, Jr., D. R. Brock, J. T. Linman and W. T. Moss

With homografts of various tissues and organs, systemic histologic changes in the host animal are not usually detectable. Recently, however, in studying whole-organ homografts of the canine liver, widespread changes which were coincident with rejection were found in host organs.[6] The changes consisted of round cell infiltration and mesenchymal tissue proliferation. They were found in bone marrow, lung, kidney, and other tissue and organs which are constituents of the reticuloendothelial system.

From the information available, it was not possible to determine if these changes were part of an exaggerated host-versus-graft rejection process, or if they resulted from an attempt by the graft to repudiate the host (graft-versus-host reaction). In the present report, an attempt was made to study this point by rendering either the graft or host animal incapable of immunologic activity by preoperative ionizing irradiation.

Methods

Adult mongrel dogs weighing 15 to 20 kg. were used. The animals were dewormed and immunized against distemper and infectious canine hepatitis. Using a previously described technique, liver homografts were transplanted with normal anatomic reconstruction of vena cava and portal veins.[5] The host spleen was removed. Blood chemistry and hematologic data were obtained before and after operation. Autopsy was performed promptly after death.

Ionizing irradiation was given either to the prospective recipient animal or to the dog from which the liver homograft was to be obtained. All animals were given 1,400 r total body irradiation in a single dose. The dose was measured on the surface of the dog, including back scatter; 700 r to the right lateral side, 700 r to the left lateral side. Factors were 280 kv. hvl., 1 mm. Cu, 20 ma, target dog distance 167 cm. Dose rate at the surface of the dog was 14.2 r per minute. The dose to the center of the dog's abdomen was calculated to be 900 to 1,000 r.

Results

Irradiation of Host.— Six recipient dogs were given 1,400 r. Eighteen to 40 hour later total hepatectomy was performed and a liver transplanted from a normal dog. All animals died within 36 hours from diffuse gastrointestinal hemorrhage or hemorrhagic pneumonitis. The combination of operative trauma and irradiation appeared to preclude even temporary success.

Irradiation of Graft.— Twelve donor dogs were given 1,400 r. Eighteen hours later, their livers were transplanted to normal hosts. Six dogs died within 30 hours of hemorrhagic gastroenteritis. The other 6, which constitute the bulk of the report, recovered and were studied until their later death.

Survival and Clinical Behavior: The 6 dogs which survived the immediate effects of surgery lived for 5, 6, 8, 9-1/2, 13, and 13-1/2 days. Clinical behavior was similar to that previously observed with the use of nonirradiated grafts[5,6] and was characterized by fever, eventual lassitude, cessation of oral intake, and jaundice.

Blood and Urine Studies: All dogs had progressive elevation of serum bilirubin and alkaline phosphatase after the fifth and sixth days. Hypoglycemia was frequent late in the course. There were no changes in blood urea nitrogen. After 4 to 6 days, bile was detected in the urine. Before death, all 6 dogs had rises in the white count to as high as 45,000 per cubic millimeter.

Gross Findings: Findings were similar to those obtained with use of nonirradiated grafts.[5] The livers were usually large, firm, and either pale or mottled. Ascites was variable. In 2 of the animals, there was no obvious cause of death other than graft rejection. In the other 4, massive hemorrhage, ileal intussusception, hemorrhagic ileitis, or duodenal perforation were found and could have been the cause of death. Host lymph nodes were enlarged throughout.

Microscopic Findings: In the animals living 5 days or less, there were no mononuclear infiltrates in the livers. Structure was well preserved. In 6 days, well-defined diffuse and focal infiltrates of plasma cells and lymphocytes were evident (Fig. 1A), and in longer surviving animals, these were very prominent. After 6 days, structural preservation varied from an intact architecture (Fig. 1A) to almost complete destruction (Fig. 1B). The primary area of hepatic parenchymal loss were central lobular and periportal (Fig. 1).

Histologic evidence of host response was found. In 5 of the 6 animals

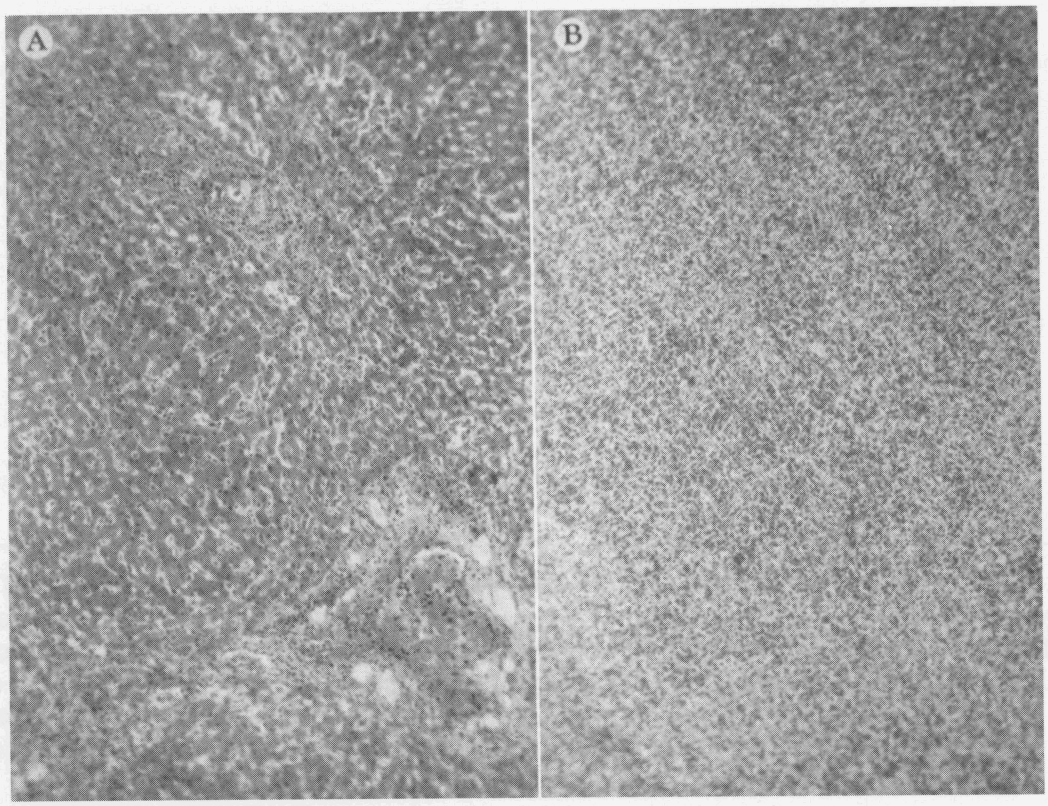

Fig. 1.— Liver in early and late stage of rejection. *A*, dog No. 6: 6 days; *B*, dog No. 9: 9 days. Reduced about 39% from mag. x100.

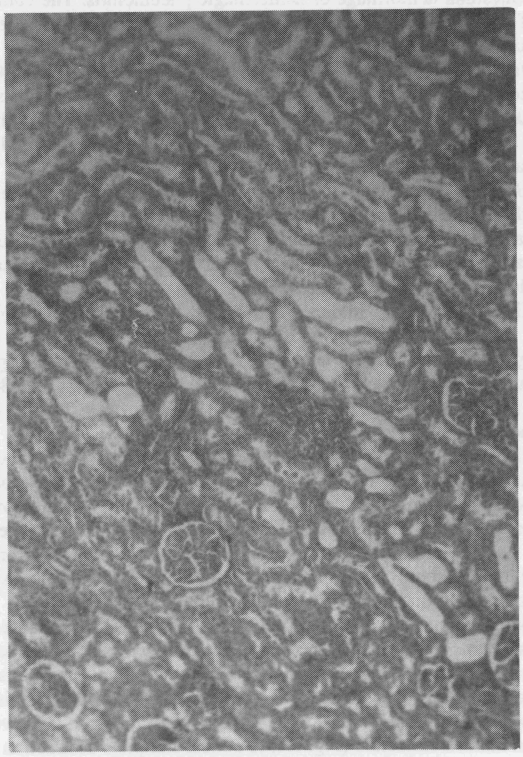

Fig. 2.— Lung from dog No. 13, after 18 days. Note proliferation in alveolar septa. Reduced about 39% from mag. x250.

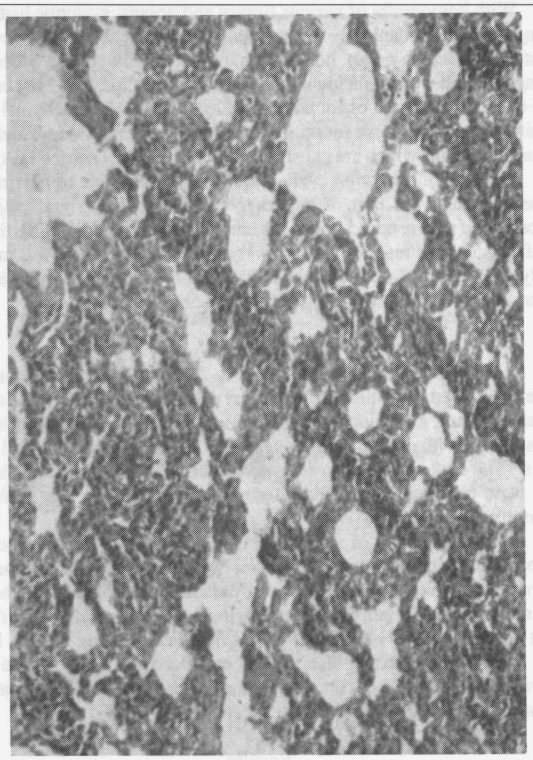

Fig. 3.— Kidney from dog No. 16, after 13-1/2 days. Cellular infiltrate in central part of illustration is focal. Reduced about 39% from mag. x100.

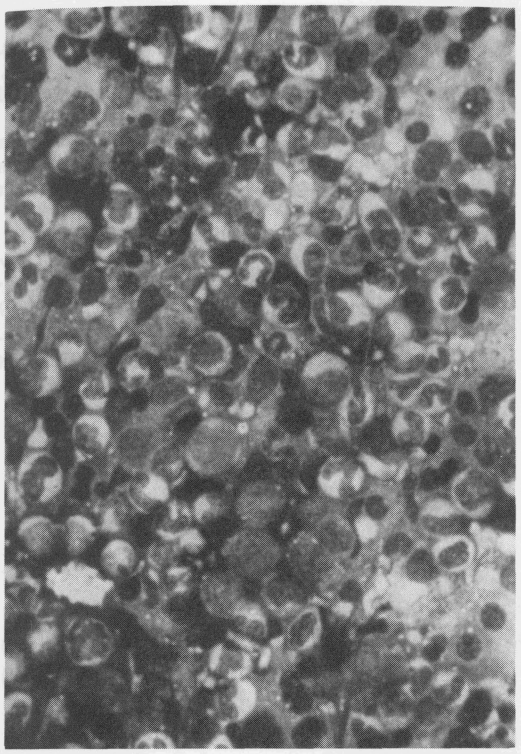

Fig. 4.— Bone marrow after 8 days from dog No. 13 showing increased numbers of plasma cells and lymphocytes. Reduced about 41% from mag. x430.

Fig. 5.— Lymph node from dog No. 13, an 8 day survivor. Washed out follicular pattern is discernible. Reduced about 39% from mag. x100.

surviving 5 days or more, proliferative changes were found in the alveolar septa (Fig. 2). In the kidneys, there were focal aggregates of mononuclear cells (Fig. 3). Bone marrows were consistently altered. Slight to marked increases in plasma cells, lymphocytes, and reticulum cells were evident in the marrow of each animal. An example of such a change of intermediate severity is shown in Figure 4.

In all the dogs, there was evidence of increased cellular activity in the mediastinal and mesenteric lymph nodes. The number of plasma cells were increased, especially in the medullary sinuses. In half of the animals, there was distortion or absence of the follicles (Fig. 5). In the other half, the basic lymph node architecture was preserved. The gastrointestinal tract, pancreas, and heart showed variable changes of the type previously related, at least in part, to the trauma of surgery.[5,6] There were no changes in skeletal muscle.

Controls

Previous control studies demonstrated that the act of transplantation did not by itself result in major histologic changes in host or graft.[6] Controls in the present study were designed to determine the effect of irradiation on the histologic character of the liver. Nine animals were given 1,000-1,400 r, with the same factors of irradiation as described above, and observed until death 4 to 13 days later. In all cases, the platelet and white counts fell precipitately after 3 to 5 days.

The architecture of the liver was well preserved. Infiltrates did not occur. However, small necrotic foci with noninflammatory response were found in 4 animals. Loss of basophilic staining was inconstantly noted.

Comment

Initially, it was hoped to perform experiments in which either the liver homograft or the host received a high dose of irradiation. However, efforts at host irradiation were unsuccessful. Irradiation injury to the bowel, combined with the transient elevation of portal pressure during transplantation, resulted in irreversible intestinal injury and fatal gastrointestinal hemorrhage.

The information gained from irradiation of the hepatic graft is useful in interpreting the interaction between the graft and the host. The graft was irradiated in situ approximately 18 hours before its removal. The calculated dose to the liver was in excess of 1,000 r, enough to effect complete immunologic paralysis of the graft.[2] Having thus eliminated the possibility of a graft-versus-host reaction, the changes in both graft and host can be attributed to the antigenic stimulus of the graft and the resultant response of the host. Survival time, clinical behavior, chemical determination, and pathologic findings were similar to those previously reported in recipients of nonirradiated livers.[3,6]

The origin of the cellular infiltrate in rejected homografts has been the source of speculation and controversy for several years. It has been suggested by Simonsen[4] and Dempster[1] that the mononuclear infiltrates are of graft origin and represent a graft-versus-host reaction. In the present study, however, the histologic character of the rejected irradiated liver did not differ from that previously observed in nonirradiated homografts.[6] These observations provide support for Hume's contention,[2] derived from studies with renal and hematopoetic homotransplants, that the cellular infiltrate in homografts undergoing rejection is of host origin.

In earlier studies of nonirradiated liver homografts, various host organs displayed alterations which resembled those found in grafts undergoing early rejection. The changes consisted of fixed tissue proliferation and round cell infiltration.[6] It was not possible to be certain if they were part of the host rejection of the graft or if they represented a graft-versus-host reaction. The present study, employing immunologically immobilized hepatic grafts, provides evidence that the abnormalities in the hosts' bone marrow, lungs, kidneys, lymph nodes, and other tissues were due to an exaggerated host response to the massive antigenic stimulus of the foreign liver.

Summary

Whole organ hepatic homografts were performed after massive irradiation of the host animal or of the animal from which the graft was taken. After host irradiation, technical success with grafting was not achieved.

With irradiation and consequent immunologic paralysis of the liver homograft, survival for as long as 13-1/2 days was attained. Length of survival, clinical behavior, chemical determinations, and pathologic findings were essentially the same with the use of irradiated as with nonirradiated livers. These findings indicate that a graft-versus-host reaction is not of immediate significance even with as bulky a graft as the liver. Histologic changes in the liver graft, as well as those in various host organs, appear to be attributable exclusively to host activity in response to the antigenic stimulus of the liver.

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This important paper introduced the chilling prospect that widespread ischemic necrosis caused by an immunologically mediated vascular insufficiency could occur in liver homografts that had been well protected by azathioprine from cell mediated rejection. In ten experiments, the longest survival was 12 days.

The concept introduced by Moore may not have been completely correct mechanistically. However, the article was prophetic in that infection introduced through livers damaged by ischemia or rejection emerged before long as a central management issue.

The devastating role of infection was delineated early in the Colorado clinical experience. An experimental paper by Brettschneider et al* attempted to determine the etiology of the infections. The conclusion that the liver graft itself was a porous entry site for bacteria indigenous to the gastrointestinal tract was an early example of the so-called "translocation" of bacteria which has become recognized since in a variety of other conditions. By 1969, when a textbook on liver transplantation was completed,** the sublety of the problem was appreciated in that powerful immunosuppression was envisioned as the only way to avoid "graft mediated infection."

* Brettschneider L, Tong J L, Boose D S, Daloze P M, Smith G V, Huguet C, Blanchard H, Groth C G, and Starzl T E: Specific bacteriologic problems after orthotopic liver transplantation in dogs and pigs. *Arch Surg* 97:313-22, 1968

** Starzl TE (with the assistance of CW Putnam): *Experience In Hepatic Transplantation.* W.B. Saunders Co., Philadelphia, 1969, pp.329-47

Immunosuppression and vascular insufficiency in liver transplantation

Annals of the New York Academy of Science, 120: 729-38, 1964

F. D. Moore, A. G. Birtch, F. Dagher, F. Veith, J. A. Krisher, S. E. Order, W. A. Shucart, G. J. Dammin, N. P. Couch

Introduction

In the summer of 1958, these laboratories commenced work in liver homotransplantation. In 1959 and again n 1960,[1,2] we were able to report a technique for orthotopic transplantation of the entire liver in the dog. This technique employed bilateral, low pressure, nonoxygenated, veno-venous shunts to by-pass and decompress the venous congestion produced by inferior vena caval and portal venous occlusion during the anhepatic phase of the operation.

The clinical, biochemical, and histologic sequences of liver transplantation and rejection were defined. The process of rejection involved infiltration of the portal area with immunocytes, a rise in the blood bilirubin and alkaline phosphatase concentrations, and sudden demise at 7 to 12 days. The host cells concerned with rejection were heavily concentrated in the portal areas and around the hepatic veins with centrolobular congestion. Terminally, there was widespread liver necrosis.

Work prior to that time, conducted in several laboratories in this country, had demonstrated that multistage and partial liver transplantation were feasible.[3] Other laboratories joining in this work at the same time, particularly those under T. E. Starzl, further delineated the technical, clinical, and biochemical course of canine liver transplantation in the unmodified recipient.[4-6]

With increasing success in kidney homostransplantation under immunosuppressive chemotherapy,[7] it became evident that we should reapproach the problem of liver transplantation, studying the effect of immunosuppression on the natural course of liver rejection.

Early in our work and in that of Starzl[9] it became clear that the immunocytic infiltrate of liver rejection was readily abated by the use of azothioprine, with or without other chemical agents, such as prednisone, cortisone, or azaserine.

Because of these encouraging findings, a first attempt in human liver transplantation was justified, and was undertaken in the spring of 1963 by Starzl and his co-workers in Denver.[8] In the fall of 1963, in this hospital, we undertook the operation upon a patient suffering from extensive hepatic carcinoma. In none of these human cases has a survival of more than one month been obtained, despite the fact that the operation is a practical one technically, and that the immunocytic rejection response was virtually absent under chemotherapy.

It thus became clear that in both man and in the dog the prevention of the immunocytic rejection response and the cellular infiltrate was not synonymous with practical success. In our experience, the longest canine survivors have been in the region of 12 days. Starzl et al. have reported animal survivors at 22 to 35 days. In both series, reasonably normal liver histology was maintained where blood supply was good. Failure of survival has been associated in our animals with the development of multiple areas of focal ischemic necrosis in the transplanted liver.

The principal question in liver homotransplantation at this time is, therefore, to determine whether this hepatic necrosis is an aspect of the rejection response or whether it represents merely the vascular and hemodynamic sequellae of transplanting a very large parenchymatous organ which carries only a small arterial blood supply.

The work of Sicular et al.[10] casts some light on this problem, because in their experimental model, the liver is not essential to survival. Long-term survivors are obtained in the presence of a rejected liver. Ischemic damage is likewise seen.

It is the purpose of this communication to report briefly our recent studies in hepatic transplantation, with particular emphasis on this vascular component in the failure of the homograft.

Methods

Large mongrel dogs, donor and recipient, are prepared by the preliminary administration of oral neomycin. In about one-half of our animals azothioprine (6 mg./kg.) was given to the recipient daily for three days prior to transplantation. In all of them this drug has been used postoperatively. Homologous blood transfusions were avoided whenever possible by prebleeding the recipient three days prior to operation and banking this blood. We have noticed, as have others,[12] that the avoidance of

TABLE 1
DOG LIVER HOMOGRAFTS—IMMUNOSUPPRESSION

Number	Days survival	Highest preterminal value		Post-mortem vascular findings
		Alk. phos.	SGOT	
LT4–H	11	46.8	1415	Extensive lobar and focal ischemia; minimal arteriosinusoidal shunts.
LT6–H	3.5	19.2	2080	Extensive lobar and focal ischemia; minimal arteriosinusoidal shunting.
LT11–H	4	2.7	220	Minimal focal ischemia and arteriosinusoidal shunting.
LT12–H	7	66	1450	Massive lobar and focal ischemia; extensive arteriosinusoidal shunting.
LT14–H	4	36.6	3100	No lobar ischemia but minimal focal ischemia; minimal arteriosinusoidal shunts.
LT15–H	7	25.8	720	Minimal focal ischemia and marked arteriosinusoidal shunts.
LT17–H	4	21.0	730	Total main hepatic artery occlusion.
LT22–H*	7	15.5	55	No lobar ischemia; moderate focal ischemia; moderate to marked arteriosinusoidal shunts.
LT–23H*	7	34.8	380	Severe diffuse focal ischemia; minimal arteriosinusoidal shunts.
LT24–H*	12	62	120	Technically unsatisfactory.

* Treated by daily portal vein dibenzyline infusions.

homologous blood transfusions is a favorable factor in achieving the survival of dogs undergoing extensive surgical operations.

The transplantation operation is carried out under pentothal ether anesthesia with an endotracheal tube. The operative technique is essentially that previously described.[1,2] The chest is not entered. Shunts of tygon tubing are joined from the inferior vena cava to the jugular vein and from the portal or splenic vein to the jugular vein on the other side. The dog tolerates caval or portal occlusion very poorly. A normothermic recipient with two functionning shunts in place provides the ideal operative setting for a cold liver transplant.

The donor animal is operated upon normothermically, but the liver is cooled to the region of 15 to 18° C. just before transplantation.

The homotransplantation requires about two and one-half hours. Anatomic restoration of vascular anatomy is by end-to-end suture. The biliary shunt is by cholecystduodenostomy. The average ischemic period for hepatic arterial flow is 40 to 50 minutes and for portal flow, 40 to 60 minutes. Tris buffer is used when the shunts are closed; essentially normal blood gas analyses pertained throughout. At the close of the operation, the animal's blood pressure, pulse, urine flow, electrocardiogram, and body temperature are normal.

The spleen was removed in about half of these recipients. At the close of the operation, polyethylene cannulae were placed in the splenic vein and in the splenic artery for postoperative angiography. Post-mortem hepatic arteriograms were made using a mercury-barium mixture.

Clinical management of these animals was essentially the same as that prior to the use of immunosuppressive chemotherapy. However, in these animals, azothioprine doses ranged from 4 to 8 mg./kg.; azaserine was at doses of 0.3 mg./kg. In several of the more recent animals, large doses of cortisone (up to 300 mg./day) were used throughout the animal's course.

To avoid excessive blood sampling, it is essential to select but a few significant blood indices of hepatic function. Alkaline phosphatase, transaminase (SGOT), and bilirubin concentrations provide the best indices of bile duct activity, liver cell necrosis, and obstruction, respectively. A characteristic course has been an early elevation of the transaminase with restoration toward normal during the succeeding days. The alkaline phosphatase rises relentlessly beginning on the second or third day until one or two days prior to death when both the transaminase and the alkaline phosphatase rise precipitously. In contrast, the bilirubin remains normal or only minimally elevated throughout the course. This represents a difference from the previously reported animals without chemotherapy, in whom the portal infiltrates and rising bilirubin were characteristic.

Antibiotics (penicillin and streptomycin) were used in all animals.

Results

In Table 1 is shown a list of survival times, terminal chemical findings, and vascular changes in 10 animals. It will be noted that we have had only six dogs in this recent group that survived a week or longer. Survivors at 5 to 12 days are up and around, taking food and fluid normally, with normal behavior and good clinical conditions. In some instances, although chemical changes have been ominous, clinical condition has remained satisfactory until an hour or two prior to death.

Pathologic findings outside of the liver have been especially noteworthy. The reticuloendothelial system does not show evidence of destruction or lysis if drug doses are kept in the range indicated. The heart frequently shows an area of myocardial necrosis or calcification if the operation has not gone well, or if there has been severe hypotension. This is a common finding in dogs after long or shocking procedures. Pancreatitis is a common complication of this operation.

Pathologic findings in the liver are notable for two features: a lack of immunocytic response, and scattered areas of severe ischemic damage ranging from focal to multilobular. Both of these characteristics of the animal liver were noteworthy in the post-mortem examination of our single human case. Histologic findings in this case are compared with those in canine hepatic replants and untreated homografts in Figures 1 through 4.

Vascular injection studies showed changes in these animals best analyzed in two groups.

1. Post-mortem. Post-mortem injection studies have shown several types of change as noted in Figure 5. The two most prominent were those of "hepatosis" and areas of absent arterial filling. Hepatosis is defined clinically as a liver engorgement with a purplish dark or black color as viewed in the living. Angiographically, such livers show considerable spill

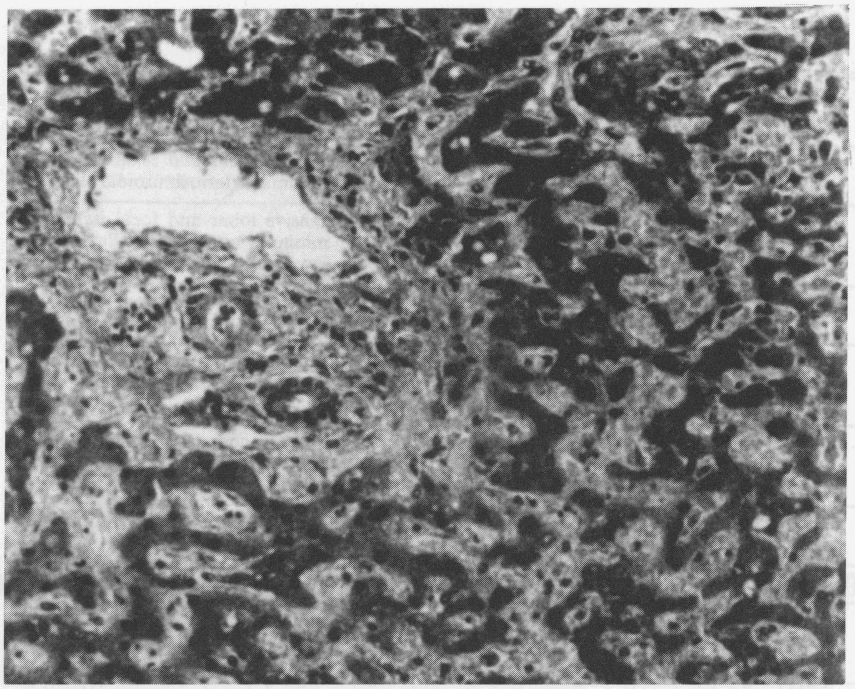

Fig. 1.— PBBH A63-281. Human whole-liver homograft on 11th post-transplant day. In the upper left field is a portal area, with the structures contained in it readily recognizable as being normal. The cellularity of the portal area is slightly increased by the presence of a small number of lymphocytes. The hepatic cells show a regressive alteration in that there is a reduction of cytoplasm and there is moderate to marked pyknosis of nuclei. The sinusoids are dilated and contain scattered mononuclear cells. As the centrolobular area is the upper right portion of the field is approached, the sinusoids become more widely dilated and hepatic cells are reduced in number and some are necrotic. The sparse cellularity of the portal area and the centrolobular area is attributed to the effectiveness of the chemical immunosuppressants employed. This contrasts with the pattern seen in the hepatic homograft on the fifth day (Figure 2) and the eighth day (Figure 3) in the unmodified canine recipient. Note: All photomicrographs (Figures 1 through 4) are at the same magnification.

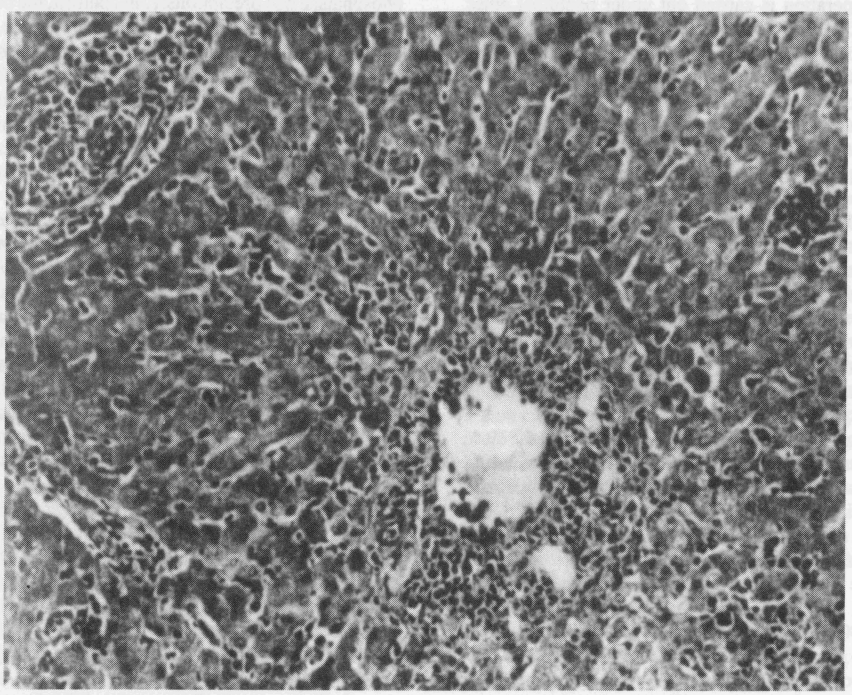

Fig. 2.— Dog X 18. Whole-liver homograft on the fifth post-transplant day. In the upper left field is a portal area with an increase in cellularity which obscures the structures in the area. Large mononuclear cells are contained in the portal area, in the sinusoids, and they increase in number as the centrolobular area is approached, which is in the center of the field. Mononuclear cells line the centrolobular vein and are concentrated around it. Where the cellular infiltration is intense, hepatic cells are lacking.

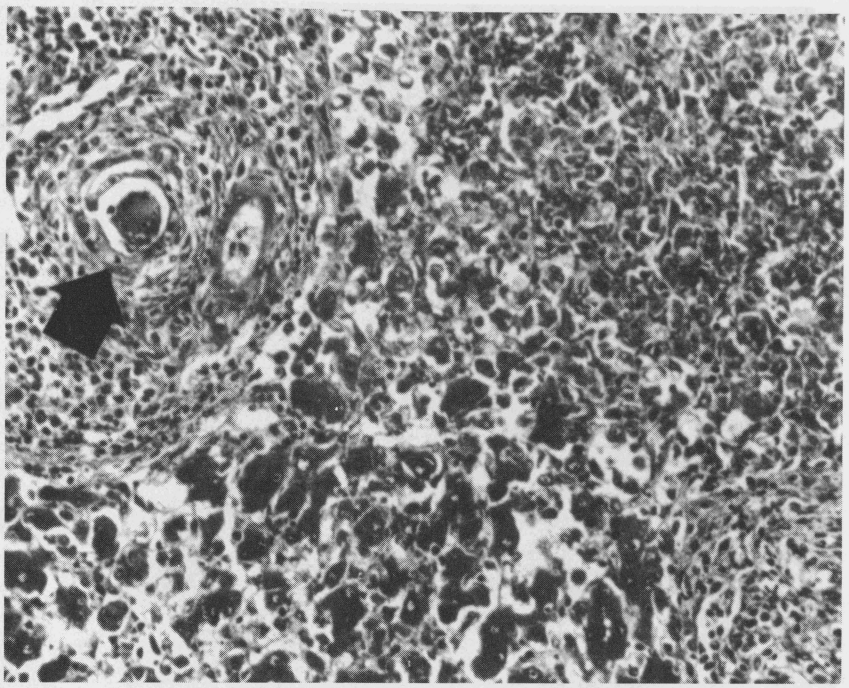

Fig 3.— Dog X 69. Whole-liver homograft on the eighth post-transplant day. The portal area shown in the upper left portion of the field is densely infiltrated with large mononuclear cells. The portal vein is obscured by the cellular infiltration. The bile duct is dilated and the epithelium is flattened (see arrow). There is a striking reduction of hepatic cells wih a collapse of the architectural pattern. In the upper right portion of the field, a centrolobular area shows hepatocellular disarray and few sinusoids are recognizable between the portal and centrolobular areas.

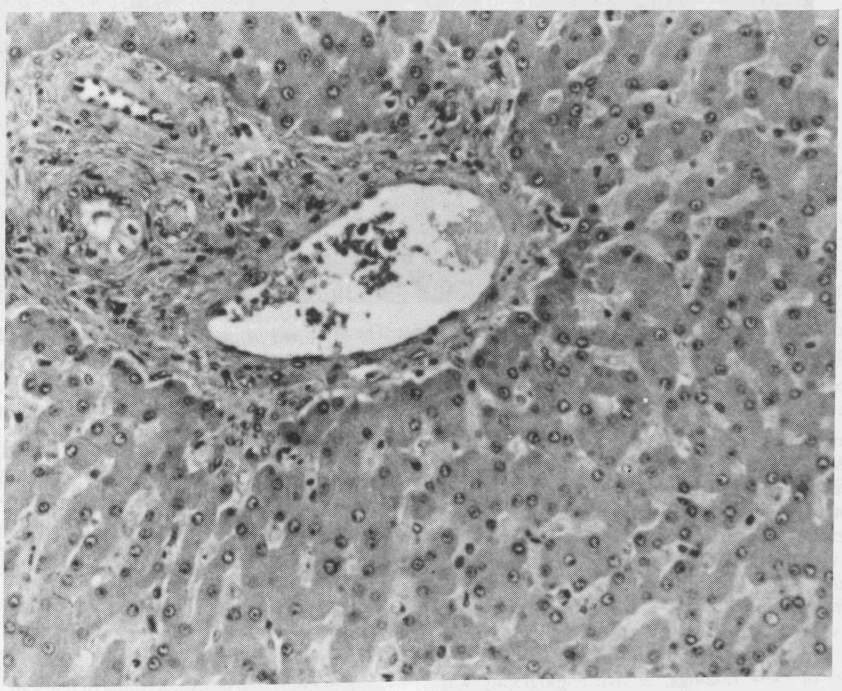

Fig. 4.— Dog X 64. Whole-liver replant on the 10th post-operative day. The portal area shows no alteration of portal vein, hepatic artery, or bile ducts. The cellularity of the portal area is slightly increased. The hepatic cells show the normal ratio of total cell size to nuclear size, and the architecture is well preserved. This Figure is introduced to show that the surgical procedure itself does not result in alterations seen in homografts.

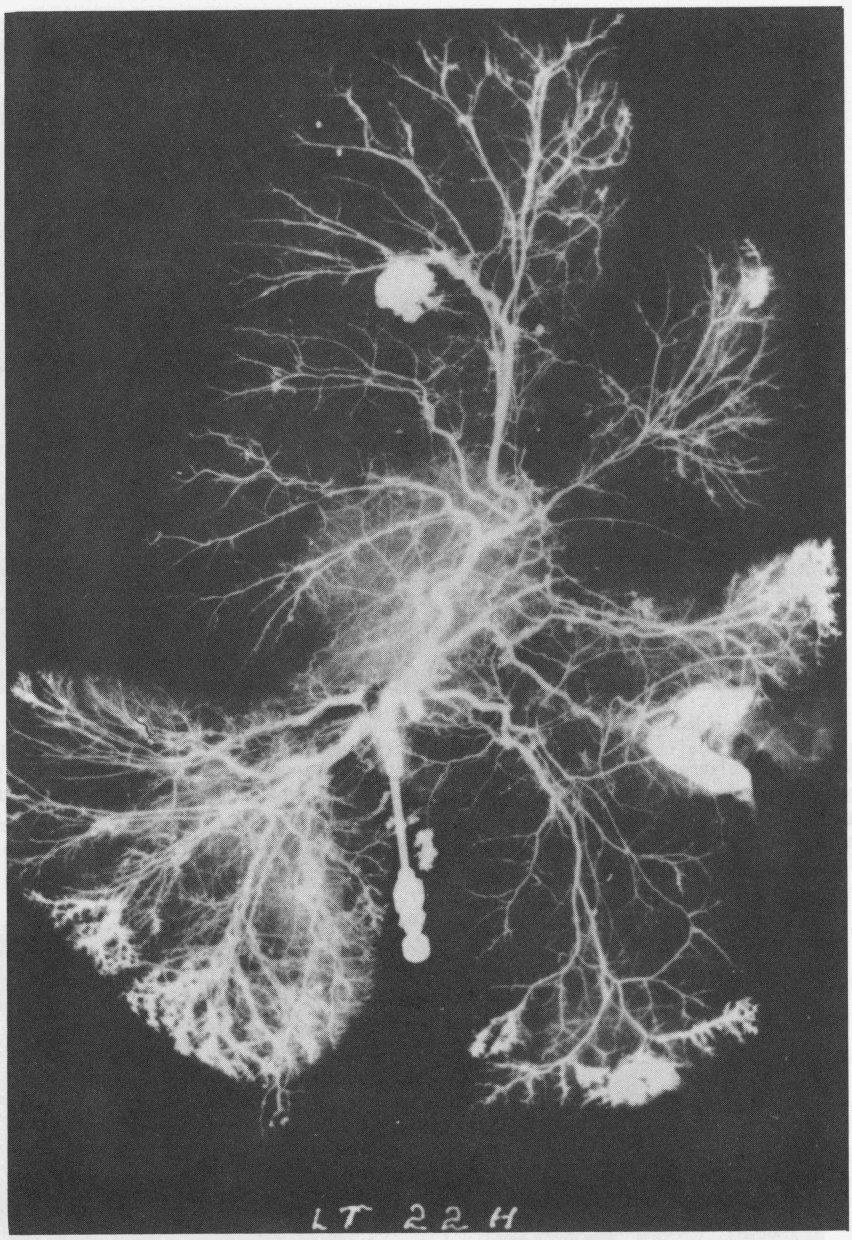

Fig. 5.— Post-mortem hepatic arteriogram. Homotransplant, seven days. The arteriosinusoidal shunts appear as grape-like clusters around the arterial branches, especially noticeable in the lower portion of this photograph. Ischemia is not marked here.

of the injection mass into parenchymatous tissue that might be termed "arteriosinusoidal" shunts. In some areas, the medium flows from the hepatic artery into the venous drainage channels. The defects in arterial filling are associated with hepatic necrosis recognizable grossly and microscopically. Serial sections have failed to identify the basis for impaired filling. There is slight perivascular cellular infiltration. The phenomenon may be vasospastic, hemodynamic, or immunologic in origin; it is our tentative interpretation that the coalescence of these areas of liver ischemia produces the extreme elevation in transaminase seen in the terminal animal.

2. In vivo. Because of these findings, *in vivo* angiography was initiated about six months ago, employed the intravascular cannulae mentioned above. Such an angiogram is shown in Figure 6.

In these films, a slight narrowing of the hepatic artery at the area of anastomosis is repeatedly noted; in some instances, it may be a severe narrowing. In two such instances, hepatic arterial thrombosis was noted at this site, in one of which occlusion occurred and this was the principal cause of death. If the anastomosis is carried out obliquely and with extreme care to avoid narrowing, it is possible to have an hepatic arteriogram during life, that does not demonstrate the site of suture.

The portal venous system has shown excellent patency and normal anatomic reconstruction with seemingly excellent blood flow. Thrombosis is rare.

To date, we have been unable to demonstrate by antemortem *in vivo* angiography, the impaired filling of portions of the arterial tree noted in the post-mortem injections. This failure to demonstrate vascular insufficiency is due to a technical limitation of the method of *in vivo* arteriography since it frequently does not demonstrate the terminal branches whose failure to

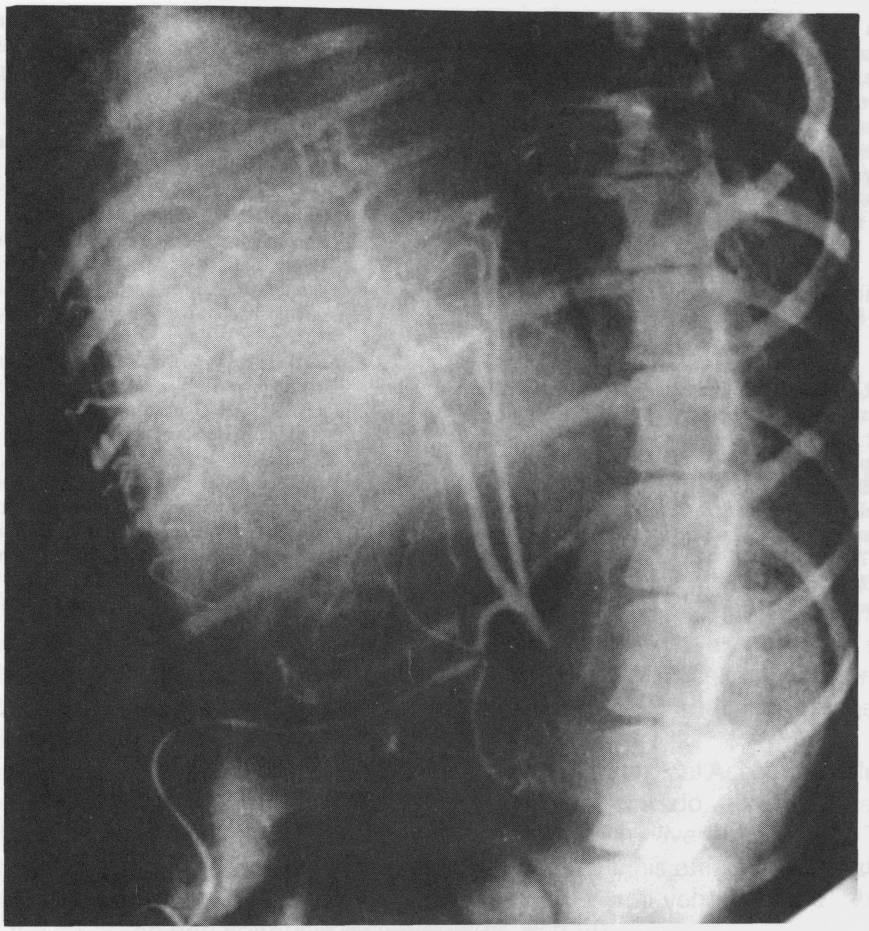

Fig. 6.— *In vivo* hepatic arteriogram. Homotransplant, four days. The anastomosis is not visible. The peripheral lobular arteries are well filled.

fill is noted at post-mortem examination.

Heparin and a vasodilating agent (dibenzyline) have been injected regionally into the liver; this step has not altered the clinical, chemical, or histologic course of the transplant.

Discussion

The work reported herein has defined rather than answered a question. The liver at death is ischemic, as shown by histologic and angiographic study. The question of outstanding importance in liver transplantation relates to the interpretation of this ischemia. Is it to be considered an aspect of rejection, or merely a by-product of arterial anastomosis, involving a rather small nutrient artery, or a very large parenchymatous organ?

The perfect control for this operation is a complete liver removal with reimplantation. This is very difficult to carry out because of the short length of blood vessels involved, and the obvious problem of temperature control in donor and recipient. The various types of autotransplantations and sham replants carried out on immunosuppressive chemotherapy have failed to demonstrate ischemic lesions as widespread or severe as those seen in the transplants. If the animal, in the course of the replantation, has a severe episode of shock, he may show both cardiac and hepatic necrosis. This is not inevitable, however, and several long-term survivors have been obtained either with autotransplants or sham replants. It is thus clear that the hepatic ischemia is not an inevitable result of the surgical procedure itself.

It, therefore, appears clear that the hepatic ischemia is, at least in part, an aspect of immunologic rejection. The nature of the operation and the hepatic arterial blood supply render the liver hemodynamically vulnerable to an ischemic lesion after the surgical procedure itself; the very high incidence of ischemic necrosis in the homotransplants and the lack of long survivors suggest that the more widespread form of this disorder is associated with an immunologic process.

The particularly unfavorable aspects of canine liver for these studies are evident. The dog liver easily becomes congested and turgid with manipulation or hypotension. The change, which we have termed hepatosis, may be related to hepatic outflow obstruction,[11] though the *in vivo* angiograms do not support the contention of outflow block. It is possible that long-term survival in experimental hepatic homotransplantation would be more easily achieved in some other species.

It is further of interest that 6-mercaptopurine, azothioprine, and azaserine are all mildly hepatotoxic drugs. Azothioprine is given to the animal by mouth and the portal concentration is probably much higher than that obtained in a systemic circulation. One may, therefore, raise the possibility that a superior form of immunosuppression for hepatic homostransplantation might be found with a drug that is given systemically, and is much less hepatotoxic. The combination of a parenteral immunosuppressive agent with local radiotherapy is likewise an appealing combination.

Conclusions

(1) Immunosuppression, using azothioprine, azaserine, and prednisone, readily abates or aborts the immunocytic rejection response in hepatic homotransplantation.

(2) This favorable development is not associated with practical clinical survival, either in the dog or in man.

(3) In both species, the terminal findings are those of extensive hepatic necrosis, spotty in some areas, and widespread in others; this is associated with non-filling of the terminal arterial branches in the involved areas.

(4) These findings are not as prominent in the autotransplanted or sham operated liver, even though the vascular anastomoses are the same.

(5) It is, therefore, our tentative interpretation that this ischemic lesion is not wholly the result of hemodynamic alterations resulting from the operation of transplantation, but that the immune response of the host may be a contributing factor.

(6) Further investigative work must be devoted to the maintenance of an adequate arterial supply following homotransplantation of the liver, and the exploration of other modes of immunosuppression.

Acknowledgments

The authors which to express their gratitude to Miss Joan Voorhees and Mr. Paul Vernaglia for their assistance in this work.

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In this attempt to prevent liver rejection in dogs receiving azathioprine, the maximum survival in 25 experiments was only 31 days. Ten of the 25 animals died perioperatively. Although some of the other 15 animals had preservation of liver function and hepatic graft architecture, there were few reasons for optimism except for an addendum describing the long-term survival in dogs in a series of new experiments. One of these dogs lived 11-2/3 years.

At the time this paper was presented in 1964 at the American Surgical Association, five patients had been treated with liver replacement at the University of Colorado, all unsuccessfully. The repeated failures stimulated interest in the possible use of auxiliary liver homografts. The "extra" livers underwent acute atrophy, and the suggestion was made in this article that the atrophy was caused by hepatic deprivation of specific substances in the portal venous blood. This was the beginning of the so-called hepatotrophic hypothesis (cf. Part IV).

Immunosuppression after experimental and clinical homotransplantation of the liver

Annals of Surgery, 160: 411-39, 1964

T. E. Starzl, T. L. Marchioro, D. T. Rowlands, Jr., C. H. Kirkpatrick, W. E. C. Wilson
D. Rifkind and W. R. Waddell

The fate of whole organ liver homografts after transplantation to untreated canine recipients is well known.[3,9,23,25,26,34,39,41] For several days after operation, the transferred tissue is life-sustaining, but after this there is rapid functional failure. The histologic abnormalities in the rejected liver consist of infiltrates of mononuclear cells which tend to be concentrated in the periportal areas, a necrotizing arteriolitis, and dissolution of hepatic parenchymal cells with retention of a relatively normal reticulum.

In the present report, an attempt will be made to add an additional dimension by describing the behavior of canine and human hepatic homografts in recipients treated with immunosuppressive agents. Under these circumstances, a modified rejection was observed in many instances despite the absence of significant cellular invasion of the homograft. In addition, it was noted that severe hepatocyte injury often occurred in livers in which the duct system was selectively preserved or even hyperplastic. Finally, several previously unrecorded observations will be documented regarding more esoteric biochemical alterations in patients after hepatic homostransplantation. These include serial determinations of plasma or serum immunoglobulins, haptoglobins, amino acids, pyruvates, and lactates.

Methods

Types of homotransplantation. Orthotopic homotransplantation was carried out in 25 dogs, after removal of the animal's own liver.[40] The reconstructed blood supply to the revascularized homograft was essentially normal.[40] The time for transfer and complete revascularization of the cooled organ averaged 70 minutes. The effects of ischemia were minimized by perfusion of the liver with cold (10-15° C.) lactated Ringer's solution prior to its removal.[40] Internal biliary drainage was provided with a cholecystjejunostomy or cholecystoduodenostomy. Splenectomy was performed. Eleven animals died during or within three days following operation. These failures were considered to be technical,[42] and are not considered in the pathologic analysis (Table 1).

In 15 dogs, an auxiliary liver was placed in the right paravertebral gutter (Fig. 1) using a modification[42] of the method of Welch and his associates,[9,47] revascularizing the portal vein from the terminal inferior vena cava and the hepatic artery from the common iliac artery (Fig. 1) or aorta. The vascular supply to the graft was thus comparable to that of a portacaval transposition[4] in that systemic venous blood passed to the portal vein. Cold perfusion of the homografts was done with the same technic as with the orthotopic livers.[40] The period of ischemia averaged 32 minutes. Cholecystoduodenostomy was performed (Fig. 1). The spleen was removed. Postoperatively, patency of the vascular anastomoses was checked with transfemoral venous and arterial angiograms (Fig. 3). In four animals, the recipient dog's own liver was removed at a second-stage operation after 27 to 28 days, leaving the homograft as the only residual liver tissue.

Five clinical hepatic homotransplantations were performed with previously described technics.[42,44,45] The homograft was placed in normal anatomic position (Fig. 3) after removal of the recipient's diseased liver. Four of the patients (ages 29-67) had primary malignancies of the liver, and the fifth was a three-year-old child with congenital biliary atresia. The livers were obtained from cadaveric sources,[42,44] using an extracorporeal perfusion system to provide interim circulation and cooling as the organ was removed.[22] The times from donor death to revascularization in the recipient ranged from 164 to 420 minutes.[42] Splenectomy was performed in only one case (Patient 2).

Immunosuppression. Azathioprine was used for the canine orthotopic experiments (Fig. 9), in quantities of 8-15 mg./Kg. per day for two to four days after operation and 2-10 mg./Kg. per day thereafter. The highest dose possible was selected which did not cause leukopenia. In three cases, this was supplemented after four to 12 days with a short course of subcutaneous prednisolone, administering 20-100 mg./day for four to ten days.

Azathioprine was used in a comparable manner for canine auxiliary homotransplantation studies (Fig. 4). In six of the 15 dogs, 100 mg./day subcutaneous prednisolone were also started on the day of operation, with subsequent reductions in the dose every two days to 50, 25, 10 and 0 mg.

The use of azathioprine was similar in the clinical cases (Fig. 11, 12). Prednisone was used (100-200 mg./day) within one-half to three and one-half days after operation in Patients 2, 4 and 5, and started before operation in Patient 3. This drug was continued until death. Intravenous actino-mycin

TABLE 1. *Pathologic Findings in Auxiliary Canine Homografts*

No.	Time of Biopsy or Autopsy (days)	General Architecture	Intrahepatic Hepatic Artery	Intrahepatic Bile Ducts	Perivenular Necrosis Central V.	Immunocytes	Cause of Death
			Only Hepatic Artery Open				
AHH 1	30	Good, central necrosis, moderate	Medial and intimal thickening	Well preserved decreased number	Moderate	Periportal, severe	Hepatic inficiency after removal of own liver (28 days)
AHH 2	28	Fair, central necrosis, moderate; generally vacuolated hepatocytes	Medial and intimal thickening	Well preserved	Moderate	Periportal, severe	
	72	Poor, only few hepatocytes remain	Severe medial and intimal thickening	Well preserved	Severe	Periportal, severe; general, moderate	Sacrificed
*AHH 7	28	Good, central necrosis, slight	Medial thickening, Focal intimal necrosis	Columnarization, hyperplasia, and bile stasis	Slight	Periportal, moderate	
	65	Fair, central and scattered necrosis, moderate	Intimal and medial thickening and focal necrosis	Hyperplasia and bile stasis	Moderate	Periportal, severe; general, slight	Sacrificed
*AHH 9	27½	Good, some vacuolated hepatocytes	Focal intimal and medial necrosis	Columnarization	None	None	Hepatic insufficiency after removal of own liver (27 days)
*AHH 10	19	Poor, diffuse loss of hepatocytes	Medial thickening, focal medial and intimal necrosis	Columnarization, bile stasis	Severe	Periportal, moderate	Pneumonia
AHH 14	33	Poor, central necrosis, severe	Medial thicking and focal necrosis	Columnarization	Severe	Rare	Not known
			Portal Vein and Hepatic Artery Open				
AHH 3	26	Excellent, central necrosis, slight	Medial thickening	Columnarization, bile stasis	Moderate	Periportal, slight	After angiogram
AHH 4	29	Fair, central necrosis, moderate; general hepatocyte vacuolization	Medial and intimal thickening	Normal, decreased number	Moderate	Periportal, moderate	Hepatic insufficiency after removal of own liver (27 days)

TABLE 1. *Continued*

No.	Time of Biopsy or Autopsy (days)	General Architecture	Intrahepatic Hepatic Artery	Intrahepatic Bile Ducts	Perivenular Necrosis Central V.	Immunocytes	Cause of Death
AHH 5	29	Good, central necrosis, slight, focal periportal necrosis	Medial thickening	Columnarization	Slight	Periportal, moderate	Hepatic insufficiency after removal of own liver (28 days)
AHH 6	27	Good, central necrosis, moderate; focal hepatocytes vacuolization	Focal medial necrosis, intimal thickening	Columnarization	Moderate	Periportal, slight	After angiogram
*AHH 8	13	Poor, central necrosis, severe; periportal necrosis, severe	Intimal thickening, focal medial necrosis	Columnarization, bile stasis	Severe	None	Hepatitis own liver
*AHH 11	24	No sections					After angiogram
AHH 12	20	Excellent, central necrosis, slight	Intimal and medial thickening; focal medial necrosis	Normal	Slight	Periportal, slight	After angiogram
*AHH 13	21	Poor, central necrosis, severe	Medial thickening	Well preserved, bile stasis	Severe	Periportal, slight	After open liver biopsy
AHH 15	45	Good, central necrosis, slight; moderate periportal necrosis	Medial thickening	Hyperplasia	Slight	Periportal, moderate	Sacrificed

* Received eight day course of prednisolone, starting on day of operation. See text.

C (200-400 micrograms/day) was administered every two to five days. In one case (Patient 2), 10 mg./day azaserine was given intravenously for two days after operation.

Studies of function. Numerous liver function studies were obtained. Analytic methods will be documented only for those determinations considered in detail in the results. Bilirubin content of serum, urine, and T-tube drainage was measured by the method of Malloy and Evelyn,[19] the one-minute reading being taken as the *direct* (conjugated glucuronide) fraction, and the 30-minute reading as the total bilirubin. Serial serum alkaline phosphatases were measured by the Bodansky method. Serum glutamic-oxalacetic acid transaminase (SG-OT), serum glutamic-pyruvic acid transaminase (SGPT), lactic acid dehydrogenase (LDH), and isocitric acid dehydrogenase (ICD) followed similar postoperative patterns and, therefore, only the results of the SGOT determinations will be described.

Plasma sugars were analyzed colorimetrically on an autoanalyzer. Plasma pyruvates and lactates were analyzed by the metods of Friedman and Haugen[7] and Barker and Summerson,[2] respectively. Venous blood for lactate and pyruvate determination was collected without stasis in a tube containing 20 mg. potassium oxalate and 15 mg. sodium fluoride. The tube was immediately placed in an ice water slush at 0° C. and taken to the laboratory where the cellular elements were removed by centrifugation at 3,000 RPM for 10 minutes. The plasma thus obtained was then frozen until the time of definitive analysis.

Plasma protein fractionation was determined by paper electrophoresis and fibrinogen was measured by the technic of Ratnoff and Menzie.[33] Quick one-stage prothrombin times were obtained. Analysis of serum and urinary amino acids is referred to in the results section. A detailed account of changes in euglobulin lysis times has been published elsewhere.[45]

Haptoglobin types. In Patient 5 of the clinical series, the serum haptoglobin genotypes of the donor and recipient were determined by vertical starch gel electrophoresis.*[35] The wells were charged with a solution consisting of 15 parts of undiluted serum and one part of 10 per cent hemoglobin solution. After electrophoresis for 18 hours, the gel was divided into 2-3 mm. slices and stained with 0.1% o-tolidine in acetic acid. The hemoglobin binding proteins appeared as blue bands.

Serum immunoglobulins. In addition, each of the three classes of immunoglobulins (gamma globulins) was quantitatively estimated for Patient 5 with a modification[5] of the immunochemical diffusion method of Huntley.[14] Commercially prepared** goat anti-human gamma$_2$ globulin, anti-human gamma$_{1M}$ globulin and anti-human gamma$_{1A}$ globulin were each incorporated into separate volumes of 1% agar in 0.85% saline (buffered with veronal, pH 7.4, μ 0.15) in a concentration of 2:1. The agar, containing the heterologous antibody was drawn into the lower portions of

* Hydrolyzed starch was the product of Connaught Medical Research Laboratories, Toronto, Canada.

** Hyland Laboratories, 4501 Colorado Blvd., Los Angeles, California.

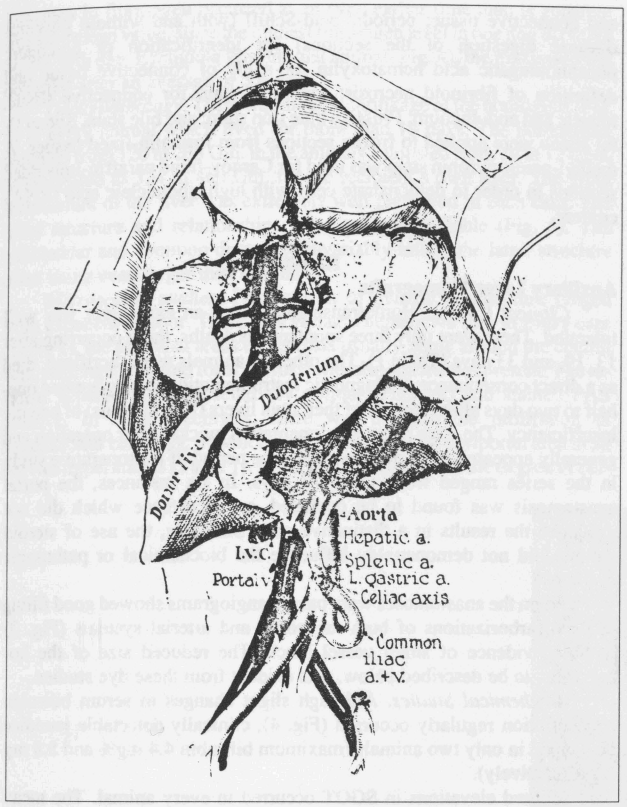

Fig. 1.— Auxiliary liver homotransplantation in dogs. Note that the reconstituted portal blood supply is from the inferior vena cava. Cholecystoduodenostomy is performed.

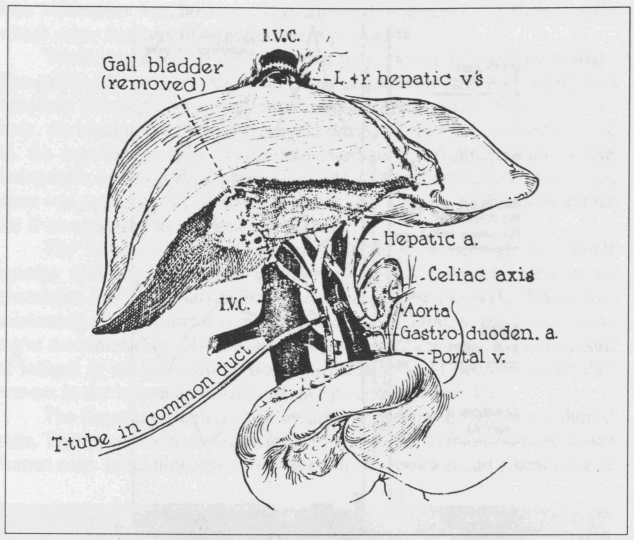

Fig. 2.— Completed clinical homotransplantation. The reconstruction is anatomically normal. The T-tube is placed through a stab wound in the recipient common duct, rather than through the anastomosis as shown.

a series of silicone-coated capillary tubes, which were sealed and supported vertically. An aliquot of the patient's serum was layered over the agar, and a precipitin band formed which migrated slowly through the agar. Tubes containing anti-gamma$_2$ globulin in the agar and overlaid with the patient's serum were allowed to stand for 24 hours. Tubes containing anti-gamma$_{1M}$ and anti-gamma$_{1A}$ stood for 72 hours. The distances traversed by the precipitin bands during these standard reaction times were measured. The level of each class of immunoglobulin in each serum sample was determined from standard curves. These curves were prepared simultaneously using pooled normal human serum, to demonstrate the relationship between distance traversed by the precipitin band and the log$_{10}$ of the con-

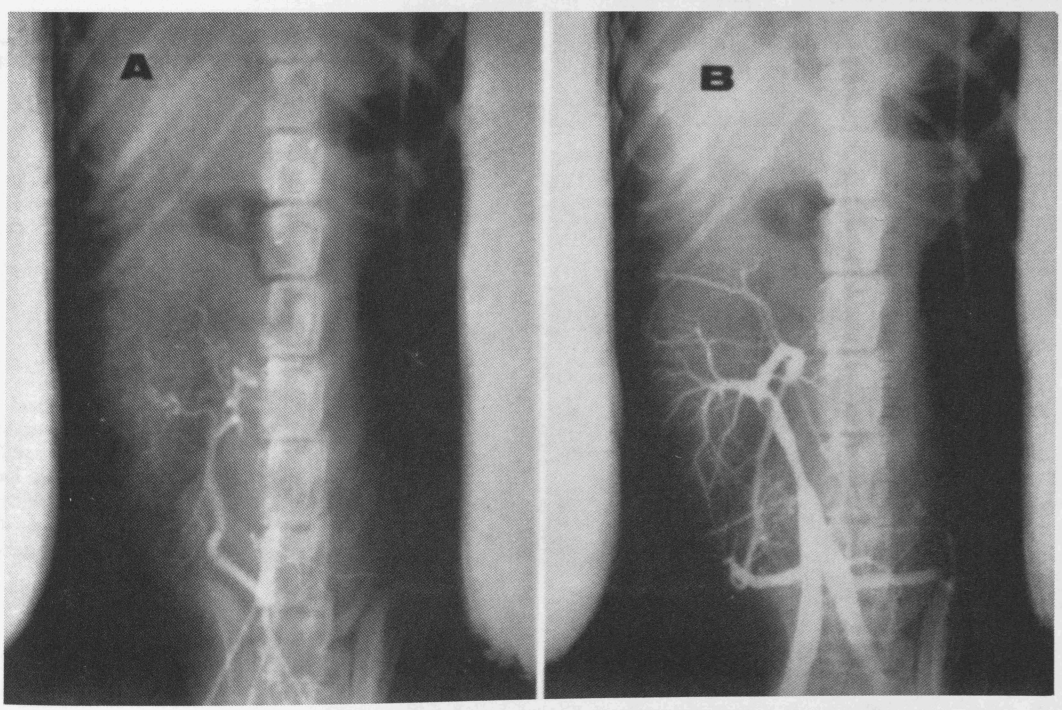

Fig. 3.— Angiographic studies of auxiliary liver homograft (AHH 6) 27 days after transplantation. A. Hepatic arterial supply B. Venous supply. Note excellent filling of small ramifications of both systems.

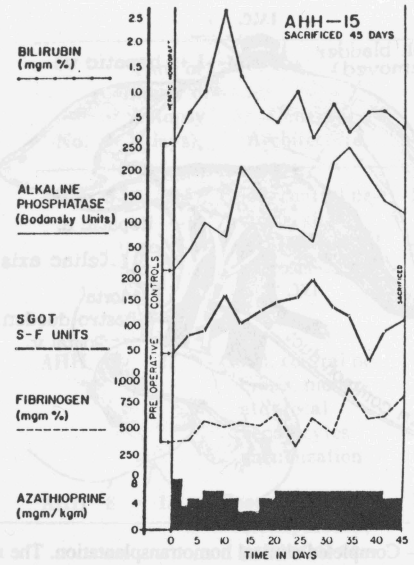

Fig. 4.— Course of dog with an auxiliary hepatic homograft. Azathioprine was the only immunosuppressive agent used. Note the irregular postoperative rises in alkaline phosphatase, SGOT and fibrinogen. The slight bilirubinemia depicted was uncommon.

centration of the respective immunoglobulin.

Pathologic studies. Autopsies were performed as promplty as possible after death. In most animal experiments, tissues were obtained immediately or within a few hours. Delay between death and necropsy in the human cases was one-half, six, 12, 12, and 16 hours. Specimens were fixed in formalin and Carnoy's solution.

A variety of tissue stains were employed for study of the livers using the technics summarized by the Armed Forces Institute of Pathology.[20] The following histologic sections were prepared from formalin fixed and paraffin imbedded blocks: hematoxylin and eosin for general architecture; silver impregnation for reticulum; Verhoeff-van Gieson method for elastic and connective tissue; periodic acid-Schiff (with and without previous diastase digestion of the sections) for identification of glycogen; phosphotungstic acid hematoxylin for study of connective tissue and definition of fibrinoid necrosis; trichrome stain for connective tissue, muscle and endothelium; Prussian blue iron stain; and bile stain. Sudan IV fat stains were applied to frozen sections from formalin-fixed tissues. A methyl-green-pyronin stain was used for Carnoy-fixed paraffin-imbedded sections in order to demonstrate cells with high ribonucleic acid (RNA) content.

Results

Auxiliary Liver Homografts

Clinical Course. Homotransplantation of an extra liver was well tolerated. There were only three spontaneous deaths, these occurring after 13, 19, and 33 days (Table 1). The other 12 animals were sacrificed, died as a direct consequence of diagnostic instrumentation, or succumbed one-half to two days after removal of their own livers (four animals) of hepatic insufficiency. The dogs resumed alimentation quickly after operation and generally appeared to be quite normal. The period of postoperative study in the series ranged from 13 to 72 days. In six instances, the portal anastomosis was found to be occluded, an occurrence which did not influence the results in a distinctive way. Similarly, the use of steroid therapy did not demonstrably influence the biochemical or pathologic findings.

When the anastomoses were patent, angiograms showed good filling of distal arborizations of both the portal and arterial systems (Fig. 3) without evidence of small vessel block. The reduced size of the homografts, to be described below, was evident from these dye studies.

Biochemical Studies. Although slight changes in serum bilirubin concentration regularly occurred (Fig. 4), clinically detectable jaundice developed in only two animals (maximum bilirubin 4.4 mg% and 5.8 mg %, respectively).

Marked elevations in SGOT occurred in every animal. The mean preoperative values were 27.8 ± 2.3 (S.E.) S-F units. Postoperative, the peak values were 293 ± 56.9 (S.E.) S-F units after 12.7 ± 2.1 (S.E.) days. In some instances, the SGOT rises occurred remittently (Fig. 4).

The most striking and sustained changes were in serum alkaline phosphatase. From control levels of 2.2 ± .1 (S.E.) Bodansky units, the postoperative alkaline phosphatases rose to 203 ± 36 (S.E.) after 18.3 ± 2.4 (S.E.) days.

Preoperative plasma fibrinogens were 301 ± 34 (S.E.) mg%. Postoperatively, these rose to 564 ± 34 (S.E.) mg% after 7.6 ± 2.6 (S.E.) days. The

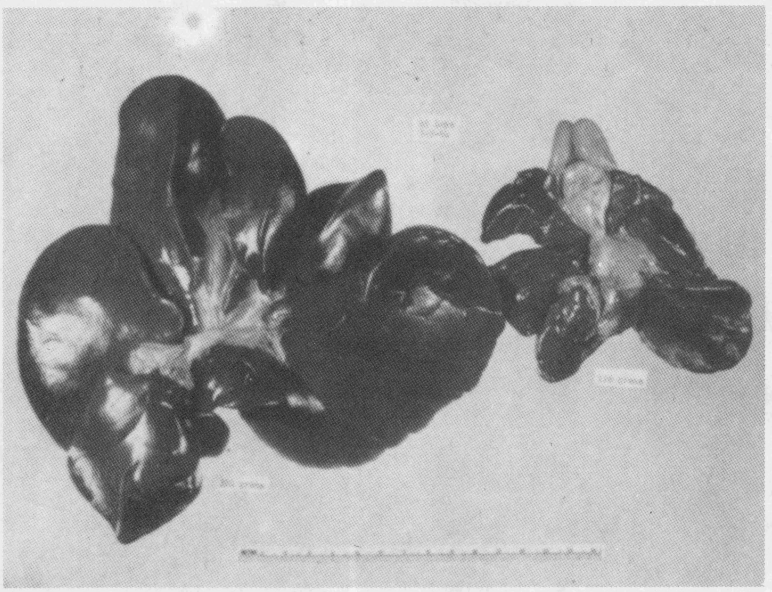

Fig. 5.— The auxiliary homograft (right) and the recipient dog's own liver (left) in experiment AHH 15. Note the well preserved but dimensionally reduced general structure of the homograft. The gallbladder did not shrink proportionately. The specimens were obtained 45 days after transplantation.

increases in fibrinogen occurred at an even earlier time than is apparent from the mean value, since the highest fibrinogen level in one dog occurred on the 34th day. Excluding this unusual animal (Fig. 4), the maximum rise occurred after an average of 5.6 days.

Gross pathology. There was marked shrinkage of the homograft (Fig. 5) in every animal which lived for more than 13 days. The homografts weighed from 120 to 265 Gm. in the animals autopsied from 19 to 72 days, as compared to 324 to 460 Gm. for the recipient's own liver. The gross architecture of the liver was extremely well preserved in each case. The lobar structure and relationships were quite identifiable (Fig. 5). The gallbladder and common duct were invariably intact, the latter structure often being even larger than normal.

Microscopic studies. Preservation of general architecture ranged from excellent to poor. There was variable hepatocyte loss in every case (Table 1), the principal areas of necrosis usually being around the central vein, although scattered focal necrosis was also sometimes present. The residual hepatocytes were relatively normal (Fig. 6A) and stained PAS positive. In most specimens, there was considerable infiltration of mononuclear cells (Fig. 6) which were localized to the periportal area in all but two experiments (Table 1). In six animals (Table 1), the degree of cellular infiltration was, however, very minimal or absent altogether, despite which other findings of rejection were present.

There was a remarkable selective preservation of the duct system. The gallbladder was invariably an easily identifiable structure, which had excellent histologic preservation of general architecture (Fig. 7). Similarly, the common duct was minimally damaged. In many animals (Table 1), the intrahepatic duct system had undergone columnarization of the lining cells, or actual hyperplasia (Fig. 6B). In 40 per cent of the specimens, there was evidence of bile stasis (Table 1) and frequently the ducts within the liver appeared to be dilated (Fig. 6B).

The intrahepatic portal venous radicals were normal. The small arteries and arterioles had widespread changes, which were most prominent in the animals followed for the longest intervals. There was thickening of the intima and media, frequently leading to marked narrowing of the lumen (Fig. 6B). These changes usually appeared to be the result of intimal or medial proliferation, but focal areas of necrosis were also present in the intima and media of many vessels (Table 1).

The hepatic reticulum was invariably preserved, but in an altered state. There was linear compression (Fig. 8) similar to that observed in one human case. In additon, there was some fragmentation and coarsening of

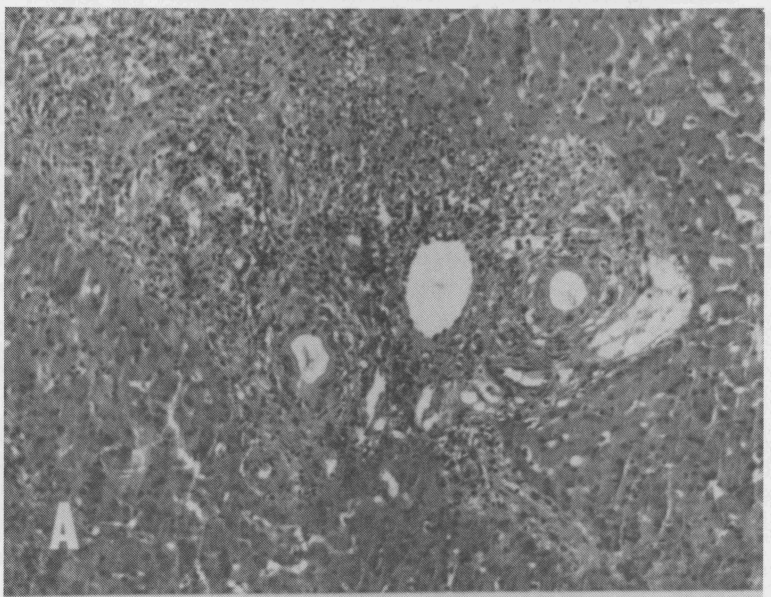

Fig. 6A.— Biopsy of auxiliary hepatic transplant at 28 days from dog AHH 7. There is increased cellularity in the portal area with good preservation of the ductal system. The adjacent hepatocytes are intact (from X 80).

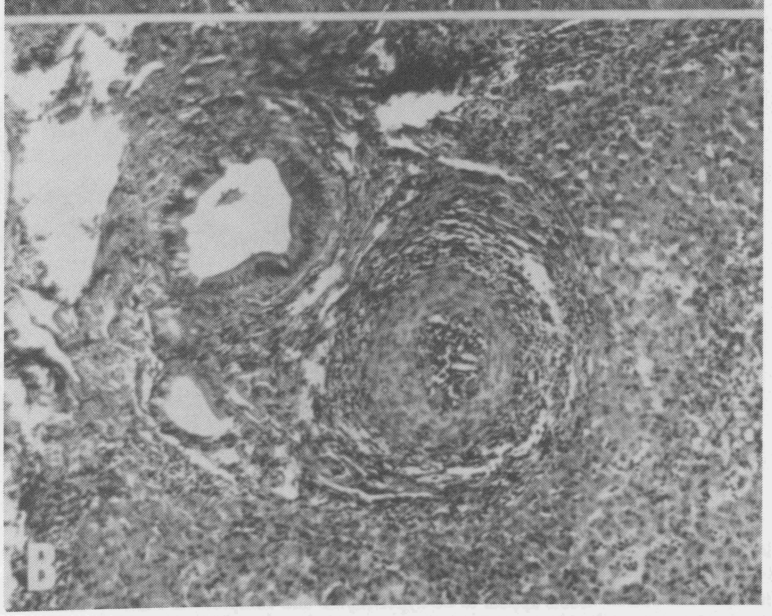

Fig. 6B.— Auxiliary hepatic transplant (AHH 7) after 65 days. The ductal system remains well preserved with columnarization and hyperplasia of the mucosal cells accompanied by moderate dilatation. The artery shows marked intimal and medial hypertrophy and a perivascular inflammatory response. Diffuse loss of hepatocytes is seen in the neighboring area. H and E (from X 80).

the individual reticulum fibrils (Fig. 8).

In two animals, serial histologic studies were obtained after one and two months, respectively (Table 1). In these dogs, the features of homograft rejection were similar in both tissue specimens, although much more marked after the longer time. The progression of the changes in the intrahepatic ductal and arterial systems were particularly striking (Fig. 6). In one of these animals (AHH2), there was destruction of almost all of the parenchyma except for the ducts.

Orthotopic Canine Homografts

Clinical course. Eleven dogs died during the first three postoperative days, almost invariably as a consequence of outflow block and/or hemorrhagic gastroenteritis. These have been reported before[42] and will not be considered further. Most of the other 14 animals made an uneventful recovery from pentobarbital anesthesia. They appeared to be healthy for the first few days after operation. Most resumed alimentation. Ultimately, all 14 died, after three to 31.5 days. The gross causes of death were pneumonia, intussusception, pulmonary embolization, or gastro-intestinal perforation (Table 2). As will be described below, the extent to which homograft rejection contributed to the mortality was difficult to assess.

Biochemical studies. It was previously established that untreated dogs developed progressive jaundice starting on the fourth or fifth day (Fig. 9) after hepatic homotransplantation.[26,41] Bilirubinemia was much less pronounced in the present treated series. The peak serum bilirubin concentrations observed were 2.8 ± 0.88 (S.E.) mg% after 9.8 ± 2.2 (S.E.) days. The onset of jaundice was frequently a terminal event (Fig. 9). In half the experiments, elevations in bilirubin were never observed. A comparison of the course of an untreated dog with that of an animal receiving immunosuppressive therapy is shown in Figure 9.

Elevations of serum alkaline phosphatase also tended to occur late in the course, the maximum rise being 22 ± 4.8 (S.E.) Bodansky units, after 8.4 ± 1.3 (S.E.) days. The very high values consistently demonstrated after auxiliary hepatic homotransplantation were not seen.

In contrast to the alterations in bilirubin and serum alkaline phosphatase, rises in serum glutamic oxalacetic acid transaminase (SGOT) tended to occur earlier, the peak values being 207 ± 21.9 (S.E.) S-F units after 5.4 ± 1.3 (S.E.) days. The early rises in SGOT, which were thought to be the consequence of ischemic liver injury during operation, were reversible to a high degree. In the preterminal period when bilirubinemia and alkaline phosphatemia were prominent, SGOT's sometimes also exhibited a secondary rise (Fig. 9), but in other animals, the enzyme levels remained relatively low despite rapid deterioration of other biochemical parameters.

Hypoglycemia, a common finding in the untreated animal[41] was never observed in dogs under treatment with immunosuppressive drugs. The lowest blood sugar ever recorded was 52 mg%.

Gross pathology. The shrinkage so characteristic of the auxiliary homografts was not observed in the orthotopic specimens. Liver weights ranged from 330 to 740 Gm. In most dogs, there was gross homogeneous preservation of the specimen, although local intrahepatic abscesses were present in two. The tissue was usually more firm than normal. The vascular supply was intact in all 14 animals under consideration for pathologic analysis. The gallbladder and common duct were intact in all but one homograft, disruption of the cholecystenterostomy having occurred in the exceptional case.

Microscopic studies. In all but one animal, there was some hepatocyte loss (Table 2), the necrosis usually being concentrated around the central vein.

There was a striking difference in the degree of round cell infiltration in the orthotopic as compared to the auxiliary hepatic homograft. Immunocytes were completely absent in four of the orthotopic livers, and cellular invasion was not prominent in any (Table 2). The characteristic hepatocyte fallout was thus present, but without consistent evidence of classical cellular rejection (Fig. 10A).

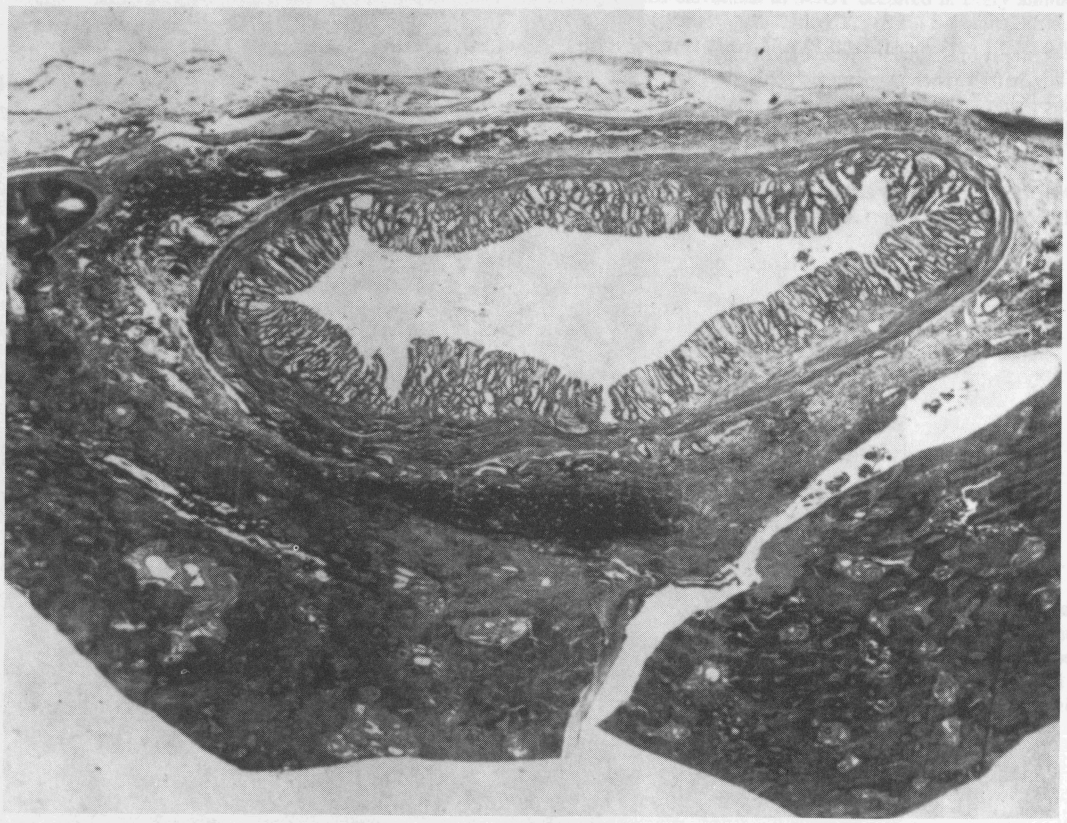

Fig. 7.— Low power scan of transplanted liver from dog AHH 7. The gallbladder is seen in the lower central portion of the figure. Note the good preservation of both the gallbladder and the intrahepatic portal tracts. The portal tracts appear to be hypertrophic and compressed. Mallory Trichrome Stain.

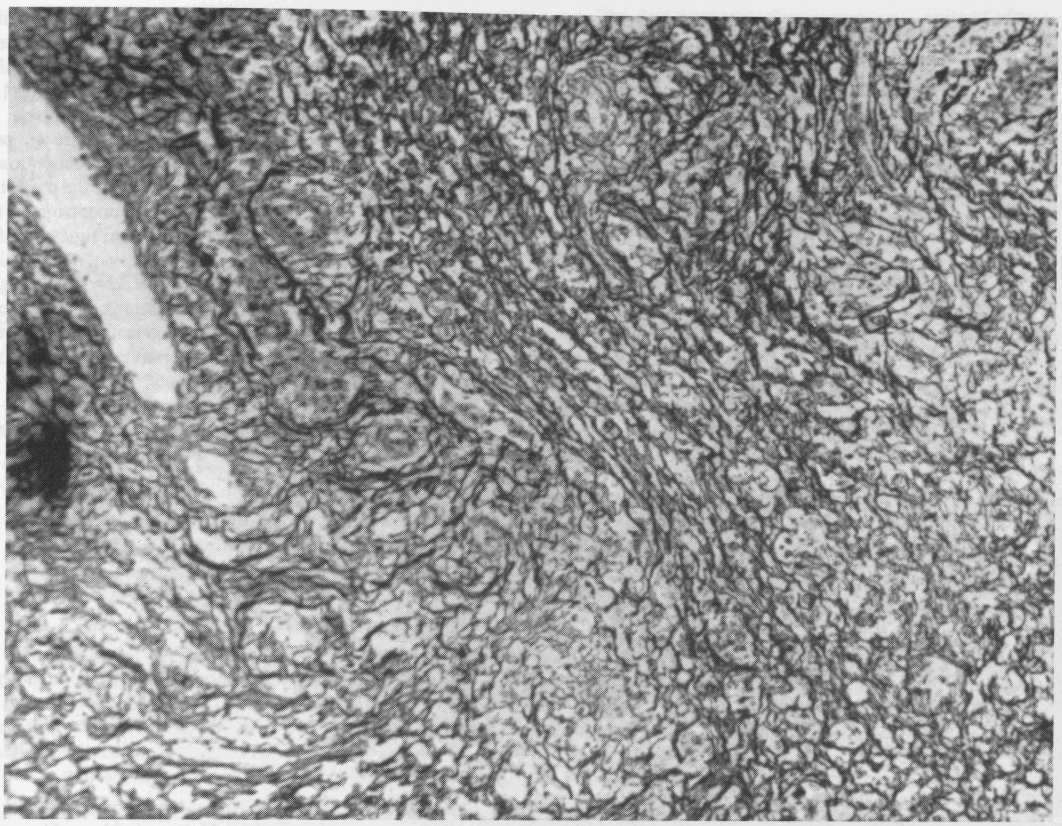

Fig. 8.— Reticulum stain of auxiliary homograft obtained after 29 days from AHH 4. The reticular scaffold is collapsed, coarsened, and diffusely fragmented (from X 80).

The intrahepatic duct system was well preserved in every case; in four of the homografts there was either columnarization of the bile duct epithelium, or actual hyperplasia, the latter finding being most prominent in the animals living for the longest time. Ductular bile stasis was present in two specimens. Hepatic arterial and arteriolar changes were present with medial and/or intimal thickening. In many instances, this appeared to be due to medial or intimal proliferation, although the smudge lesions of focal necrosis were also present. The intrahepatic portal veins were normal.

The hepatic reticulum was quite well preserved in every orthotopic homograft, although there were areas of linear compression, and some zones of fragmentation which were especially apparent around central veins in those grafts with marked hepatocyte loss (Fig. 10B).

Human Liver Homografts

Clinical course. The clinical courses of the five patients have been previously described[42,44,45] with particular emphasis on changes which occurred in the coagulation mechanism during and following surgery.[45] The first patient succumbed from operative hemorrhage. The next four died after 22, 7.5, 6.5, and 23 days. The immediate causes of death in these last cases were pulmonary embolization; pulmonary embolization and gastrointestinal hemorrhage; pulmonary embolization and congestive heart failure; and bile peritonitis due to common duct necrosis. Liver failure had an obvious role in the final outcome only in the last case, in which serious deterioration of hepatic function and a bleeding diathesis developed terminally.

Biochemical studies. In all patients surviving operation, there was evidence of moderately severe ischemic injury to the homograft (Fig. 11, 12). Serum levels of SGOT, SGPT, LDH, and ICD were markedly elevated for 24 to 48 hours. The highest SGOT's were 1,150, 990, 350, and 2,060 S-F units, respectively, in the last four cases. The serum enzyme abnormalities were rapidly reversed, however, (Fig. 11, 12) and secondary rises did not subsequently occur even in Case 5.

Preoperative jaundice was present in all but Patient 5. Postopera-

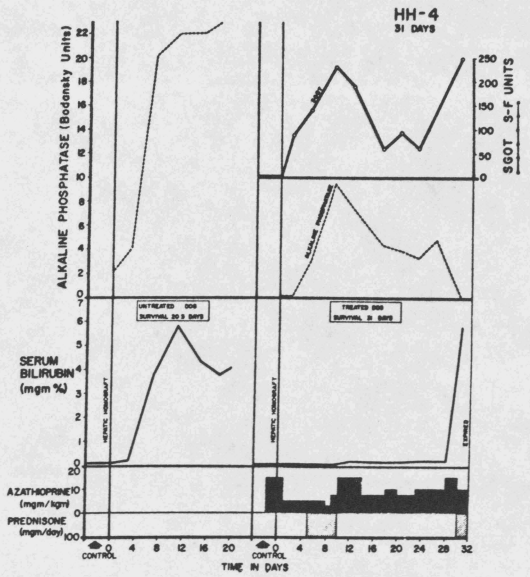

Fig. 9.— Comparison of course of untreated animal (left with that of dog treated with azathioprine and prednisone (right). The treated dog died after 31 days with a perforated gastric ulcer. Note difference of bilirubin and alkaline phosphatase in the treated compared to the untreated animal.

tively, the jaundice initially deepened in all but Patient 3, the increased early bilirubinemia reaching a peak as long as eight days after operation (Fig. 12, 13). Bile which began to issue from the common duct in all the adult cases during the operation was later shown to have a bilirubin concentration as high as 250 mg% (Fig. 13). The reversibility of the bilirubinemia was similar to that of the SGOT, except that it occurred at a later time. The early rise in bilirubin was also thought to be due to acute ischemic injury, rather than to early rejection.

Serum alkaline phosphatase was elevated preoperatively in all four of the adult cases, with a range of 12 to 35 Bodansky units. Postoperatively, these serum levels were persistently reduced to or toward normal in every instance (Fig. 11). The terminal alkaline phosphatases before death were 5.3, 6.7, 10.4, and 8 Bodansky units in Patients 2 to 5.

Prothrombin times which ranged from 40 to 100 per cent preoperatively were maintained above 25 per cent in each case except the last one. In this patient, the prothrombin time was severely depressed from the time of surgery until death (Fig. 12). In all patients, there was a slow decline in total serum protein, but severe hypoproteinemia was present only in Patient 5 (Fig. 12). Specific changes occurred in plasma fibrinogen content which have been previously described in detail.[45] These consisted of depressed fibrinogen content intraoperatively, with an almost immediate rebound which lasted for several days. In Patient 5 (Fig. 12), this sequence was seen but to a lesser degree than in any other case.

As mentioned above, progressive liver failure was present only in the last patient. In this case, restoration of liver function toward normal was interrupted on the 17th postoperative day after the patient developed acute abdominal pain. Hepatic function had been poor from the time of operation (Fig. 12), with persistently low prothrombin times, rapidly falling serum proteins, low plasma fibrinogen concentration, marked bilirubinemia, and low-voume T-tube drainage (50-150 ml../day). Although hypoglycemia did not occur, there was evidence of deranged carbohydrate metabolism. Progressive rises in serum pyruvate and lactate levels were observed (Fig. 14), beginning on the fourth postoperative day and reaching peaks of 3.4 mg% pyruvate and 73.4 mg% lactate. *Excess lactate*, calculated from Huckabee's formula,[11] was as high as 31 millimoles per liter.

Several factors which are known to influence lactate levels may have

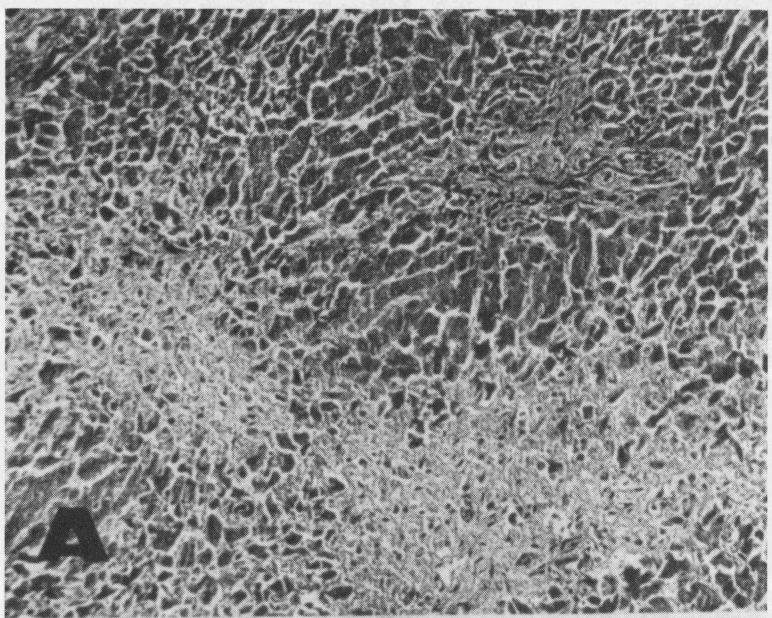

Fig. 10A.— Orthotopic canine homograft (HH 4) after 31 days. Note central loss of hepatocytes with good preservation around the portal tract. Immunocytes are absent. H and E stain (from X 80).

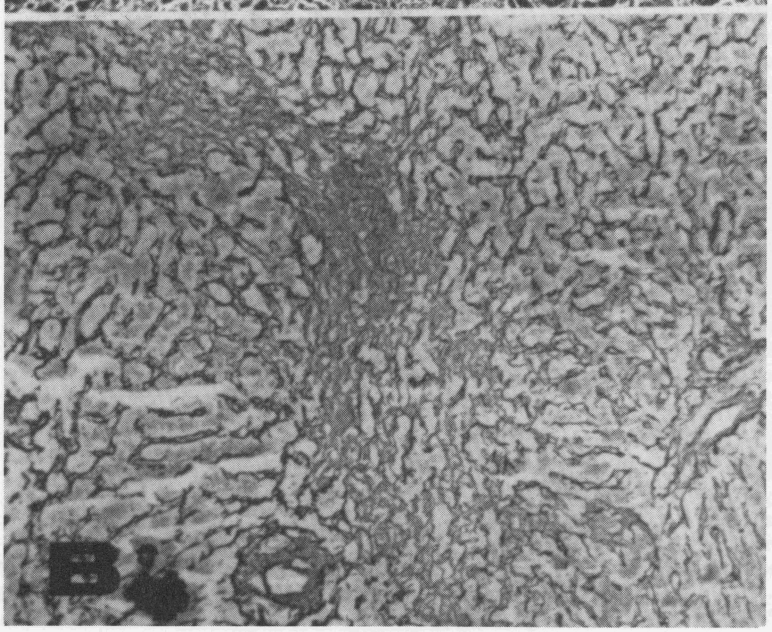

Fig. 10B.— Same dog. Note general preservation of reticulum, but with focal areas of collapse and fragmentation. Reticulum stain (from X 80).

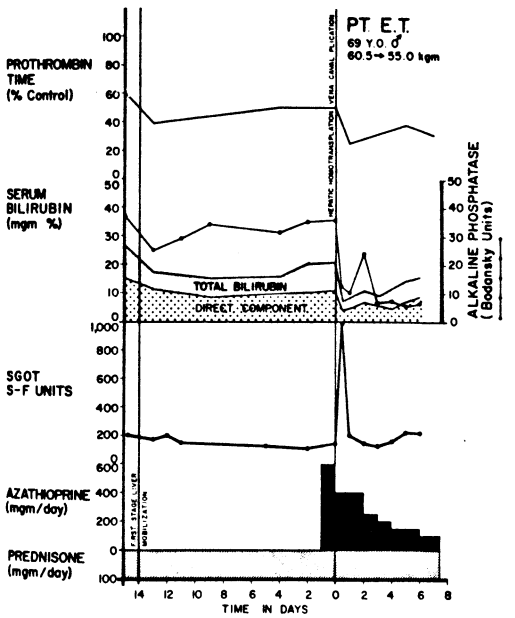

Fig. 11.— Course of Patient 3, who had an intrahepatic cholangiocarcinoma. Skeletonization of all major structures entering and leaving the liver was carried out two weeks before definitive transplantation as shown. Note early rise in SGOT after transplantation. The sudden falls in serum bilirubin and alkaline phosphatase after each operation may have been a dilutional effect due to multiple transfusions with fresh blood.

been present including steroid therapy, pain, and hyperglycemia, although none of these lead to the production of calculated excess lactate.[12] It is probable, however, that the lactic acidemia is explicable by the mechanism defined by Ballinger[1] who demonstrated the inability of the hypofunctioning liver to effectively metabolize normal or increased quantities of this substrate. Less striking elevations of serum pyruvate (highest 2 mg%) and lactate (highest 33 mg%) were documented in Cases 2, 3, and 4, but since all three patients had hypoxemia as the result of pulmonary embolization, the significance of the findings is less clear than with Case 5 in which anoxic episodes were absent until terminally.

In Patient 5, the unconjugated plasma amino acids were studied serially by means of high voltage electrophoresis.* There was a generalized increase in the concentrations of lysine, histidine, glycine, alanine, serine, valine, glutamine, and taurine.

Haptoglobin Studies. The haptoglobins constitute a population of serum proteins characterized by their ability to form a stable bond with hemoglobin.[17] The liver has been postulated to be a site of haptoglobin production.[28] The molecular heterogeneity of these hemoglobin-binding proteins was demonstrated by Smithies who observed that serum protein fractions from normal subjects had different mobilities when subjected to electrophoresis in a starch gel.[36] Smithies and Walker subsequently reported that the type of haptoglobin produced by an individual was under genetic control.[37] Three major types were identified and designated 1-1, 2-1 and 2-2.[35]

In Case 5, in which the donor and recipient were of differnt genotypes, haptoglobin studies were performed. Electrophoresis of the donor serum, obtained on the day of his death, revealed the haptoglobin genotype to be 2-2. The serum of the recipient in Case 5 was examined prior to the surgical procedures, between the first-stage operation and the transplantation, and on several occasions after the liver transplantation. The original haptoglobin type was 2-1.

* Amino acid determinations performed by Thomas C. Wood, Jr., medical student University of Colorado School of Medicine. Manuscript is in preparation.

The first post transplant serum was obtained after two days. The haptoglobin genotype had changed to an unmixed 2-2, the genotype of the liver donor. The haptoglobin patterns of the donor and the recipient, before and after transplantation, are shown in Figure 15.

Later in the postoperative period, demonstrable haptoglobins disappeared entirely. The 2-2 haptoglobin pattern was faint but probably present on the fourth postoperative day, but was not detectable thereafter (Fig. 16). The 2-1 haptoglobin did not reappear.

The biologic role of haptoglobins is not clear, although their possible functional significance and the way in which they are influenced by various disease states including trauma, liver disease and steroid therapy, have been the subject of a recent publication.[8] The observance of pure donor genotype in the recipient is of interest for two reasons. It provides evidence that the source of haptoglobin is hepatic. In addition, it demonstrates a type of protein synthesizing activity of the new liver. The disappearance of the new haptoglobin after four days cannot be construed, however, as being due to complete cessation of function of the homograft in view of the subsequent survival of 19 days, and because other factors are known to mask the presence of this substance.[8]

Immunoglobulins. The serum concentrations of immunoglobulins were serially determined in Patient 5, and compared to standard values derived from analysis of pooled human sera.[5]

When the recipient patient was first studied, before both the preliminary and definitive surgical procedures, the values for gamma$_2$ globulin, gamma$_{1M}$ globulin, and gamma$_{1A}$ globulin were within the normal range (Table 4). The concentration of gamma$_2$ globulin fluctuated considerably during the first three weeks after liver transplantation. Forty-eight hours before death it fell to significantly lower levels. The concentration of gamma$_{1M}$ globulin rose significantly on the fourth postoperative day. The level subsequently fell and by the end of the third postoperative week, it had returned to the presurgery value (Table 4). Gamma$_{1A}$ globulin fell immediately after transplantation and then remained relatively constant until 48 hours prior to death when the concentration fell still further. The changes in the concentration of the specific classes of antibody proteins are not as remarkable as the changes in protein fractions, as measured by electrophoresis, reported by Hume[13] and Kukral[16] after human renal and canine hepatic transplantation respectively. The isolated increase in gamma$_{1M}$ globulins is, however, of interest.

Gross Pathology. All organs appeared to be essentially normal. The weight of the child liver was 220 Gm. The weights of the four adult livers

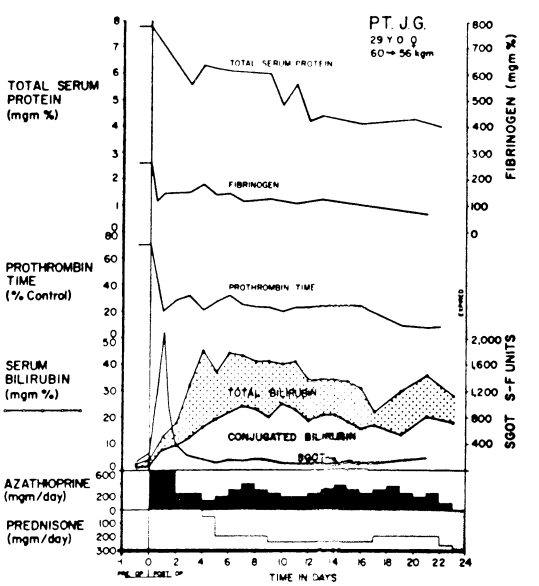

Fig. 12.— Serial chemistries in Patient 5. Note serious abnormalities in various measurements. The increase in SGOT immediately after operation was the highest observed in any case. The immunosuppressive therapy is depicted at the bottom.

TABLE 2. *Pathologic Findings in Orthotopic Canine Homografts**

Survival No.	(days)	General Architecture	Intrahepatic Hepatic Artery	Intrahepatic Bile Ducts	Intrahepatic Portal Vein	Perivenular Necrosis Central V	Immunocytes	Cause of Death
19	3	Good; central necrosis, slight	Slight intimal thickening	Normal	Normal	Slight	None	Pulmonary embolus
14	4	No Tissure						Intussusception
17	5	Excellent	Medial and intimal thickening	Normal	Normal	None	None	Pneumonia
24	7	No Tissure						
5	9	Good; scattered focal necrosis	Medial and intimal thickening, focal necrosis	Normal	Normal	None	Periportal, slight	Pneumonia, hepatic abscess
18	9	Good; central necrosis, moderate; periportal necrosis, slight	Medial and intimal thickening	Columnarization	Scattered small thrombi	Moderate	Periportal, slight	Pneumonia
22	9	Fair; central necrosis, moderate	Intimal and medial thickening	Normal	Normal	Moderate	Periportal and general, slight	Pneumonia
1	11	Fair; central necrosis, severe	Intimal thickening	Normal bile stasis	Normal	Severe	None	Pneumonia
9	12	No Tissue						Disrupted cholecystenterostomy
12**	12	Good; central necrosis, slight; periportal necrosis, slight	Medial thickening	Columnarization	Normal	Slight	Periportal, slight	Intussusception
16***	12	Fair; central necrosis, moderate	Intimal thickening	Normal	Large thrombus	Moderate	None	Pneumonia
20**	20	Fair; central necrosis, moderate; scattered focal necrosis	Focal intramural hemorrhages	Normal	Normal	Moderate	Periportal, slight	Pneumonia
3	25	Good; central necrosis, very slight	Medial thickening and fragmentation	Hyperplasia	Normal	Very slight	Periportal, slight	Pneumonia, pulmonary edema
4**	31	Fair; central and scattered necrosis, moderate	Medial thickening	Hyperplasia; bile stasis	Normal	Moderate	Periportal, very slight	Pneumonia, perforated gastric ulcer, pulmonary embolus

* Eleven additional unincluded animals failed to live as long as 72 hours. All dogs received azathioprine.
** Received supplementary prednisolone.
*** Segmental infarct present. Portal branch thrombosis.

were 1,700, 2,070, 1,720, and 1,760. Upon section, the livers seemed somewhat more pale and firm than normal. In Patient 5, there was a 2 cm. abscess in the right lobe. In the same case, there was necrosis of the distal 1 cm. of the homograft common duct.

Histologic Findings. There was extensive autolysis in the first homograft (Patient 1), which was apparently due to an excessive period of ischemia. The other homografts were generally well preserved (Fig. 17-20) with from fair to excellent over-all architecture (Table 3). The reticulum was quite normal (Fig. 18B) in all but Case 5, in which focal areas of linear compression were present (Fig. 20B). In Cases 3-5, the intrahepatic arteries and arterioles had inconstant intimal and medical thickening similar to that described above in dogs (Fig. 18A, 19).

The intrahepatic ductal system was intact in each case. In one of the homografts (Case 2), there was columnarization and hyperplasia, but this was in a liver provided by a sporadic drinker. Trichrome stains revealed the presence of minimal periportal fibrosis in this specimen. There was evidence of moderate bile stasis (Fig. 17, 20A) in two homografts. The central veins were intact but there was slight to moderate perivenular necrosis in three of the four cases surviving operation.

Immunocytes were found in the periportal area in significant numbers only in Case 3 (Fig. 18), although a few lymphocytes and plasma cells were present in Cases 2, 4, and 5 (Fig. 17, 19, 20). MGP stains were negative in all cases.

The hepatocytes were PAS positive in Cases 2, 3, and 4, but no glycogen whatever was present in the liver cells of the homograft from Case 5. In Case 3, there was a diffuse fatty metamorphosis (Fig. 18), and in Case 5 the individual hepatocytes were poorly preserved (Fig. 20). Intracellular bile pigment was present in Cases 4 and 5.

The state of preservation of liver architecture was generally much better in the human cases than in either the canine auxiliary or orthotopic homograft series.

Discussion

From the foregoing data, there can be little doubt that the vigor of the rejection process was considerably attenuated by the immunosuppressive regimens which were employed. Prolongation of survival was obtained in the animals receiving orthotopic livers. The biochemical response was considerably different from that which invariably transpires after hepatic homotransplantation in the untreated animal.[26,41] Finally, the state of histologic preservation of the homografts was far better in all three groups than could have been expected in untreated recipients. It is of interest that control of rejection in the human cases was more complete from a

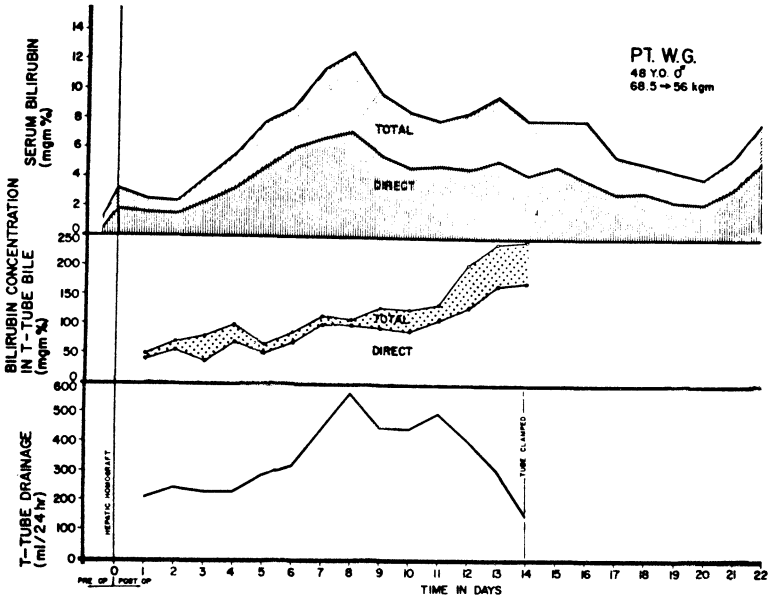

Fig. 13.— Course of Patient 2, showing relationships of serum bilirubin, T-tube drainage volume and bilirubin content of T-tube bile. Note temporary worsening of jaundice after transplantation.

histologic point of view than in the orthotopic dogs at a comparable time after operation, suggesting that the magnitude of the problem may be less in man as has seemed to be the case with renal homografts.

Despite these encouraging findings, evaluation of refinements in drug therapy has been difficult. Past efforts to potentiate hepatic homograft survival with total body irradiation have been completely unsuccessful.[38] In the present study the only agent shown to be of unequivocal value was azathioprine, and even this compound was incompletely effective. It was not possible in dogs to demonstrate with any objectivity a consistent improvement in results with the addition of prednisone to the basic azathioprine regimen. If a beneficial steroid effect is present, as has been demonstrated after canine renal homotransplantation,[21] it is probably being obscured by the many other pitfalls which make difficult the consistent attainment of a useful liver preparation.[42] Alternatively, the possibilities must be conceded that the various drugs used are not so selective in preventing hepatic destruction as they are in preventing renal homograft repudiation, that the liver is more highly antigenic and evokes a crushing immunologic response, as Greene's heterotransplantation studies would seem to indicate,[10] or that hepatic tissue is inherently more vulnerable to rejection injury.

Special attention should be directed to the influence of immunosuppression upon the pathologic features of rejection. In the untreated recipient, the most prominent findings are those of classical cellular rejection, with early and extensive invasion of lymphocytes and plasma cells[3,23,26,41]

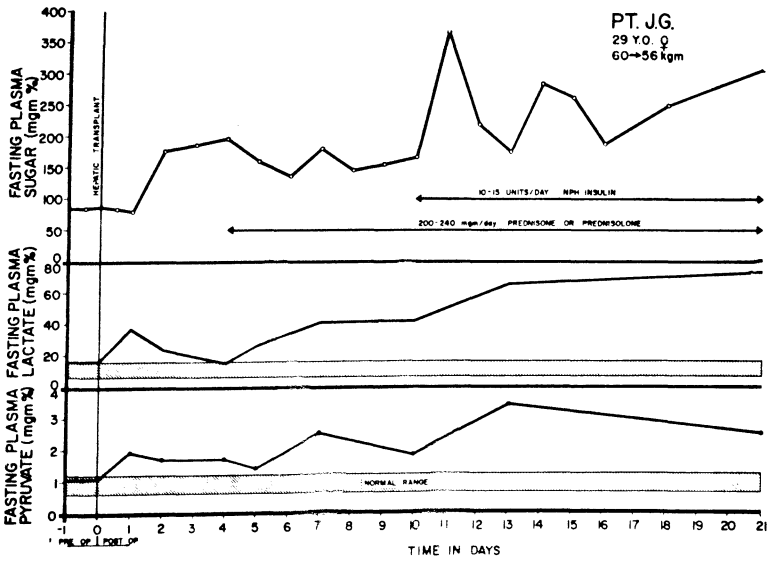

Fig. 14.— Changes in serum lactate and pyruvate in Case 5. A transient elevation occurred immediately postoperatively with subsequent decline toward normal. The secondary rise beginning on the fourth day was progressive. Note the normal to increased levels of plasma sugar. Normal ranges for serum lactate and pyruvate are indicated by the shaded bars.

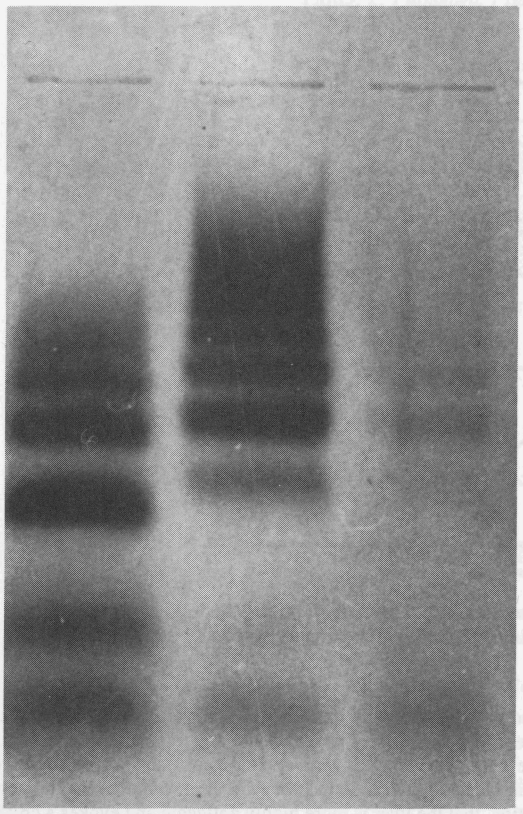

Fig. 15.— Starch gel electrophoresis showing the haptoglobin genotypes of donor and recipient in Case 5. The genotype of the recipient prior to surgery was 2-1 (left) and the donor was 2-2 (center). The donor type haptoglobin was found in the serum of the recipient on the second postoperative day (right).

which occurs coincidentally with or just before rapid disappearance of hepatocytes. Inflammatory changes in small hepatic arteries and arterioles have also been described.[3,25,38,39,41] It has been traditional to regard the cellular invasion as the primary event in the process with the small lymphocyte being the actual agent of destruction.[46]

With immunosuppression, the alterations are often strikingly different. The geographic distribution of hepatocyte dissolution is the same, being concentrated most heavily in areas around the central veins. The arteriolitic lesions are also found in various stages ranging from focal intramural necrosis to medial and intimal thickening and proliferation. But these changes may occur without significant invasion of immunocytes.

The high incidence of *non-cellular rejection* in the orthotopic canine series necessitates a re-evaluation of the mechanisms of hepatic homograft rejection, just as has been the case for similar reasons in the field of renal homotransplantation.[15,31,43] Physical contiguity of immunocytes to the parenchymal cells is apparently not a requisite.[27] Instead, there is increasing reason to suspect that humoral antibodies may play a crucial role. Sicular and his associates[34] have obtained circumstantial evidence that gamma globulin is fixed in the macrophages, bile ducts, vessels, and hepatocytes of the transplanted liver. More recently, Popper[30] reports that the greatest selectivity of antigen-antibody complexing is in the hepatocytes, a finding which could readily explain many of the pathologic findings in the presently reported series.

Whether or not the non-cellular rejection seen during immunosuppression is fundamentally different from the better known variety of tissue repudiation in untreated animals is open to question. It is possible that the primary mechanism in either case is served by humoral antibodies, and that the immunosuppressive treatment merely slows the process sufficiently to allow observational dissection of the serial events. In this concept, the

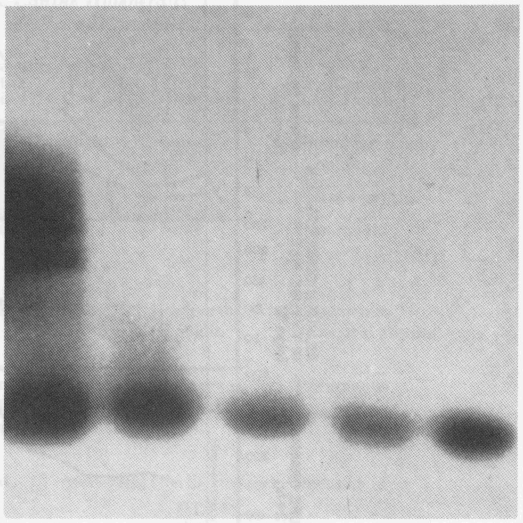

Fig. 16.— Starch gel eletrophoresis of the recipient's serum on the second, fourth, sixth, tenth and 17th post-transplant days (left to right). No haptoglobins could be found after the fourth day.

monocytic invasion of the *classical cellular rejection* could be viewed as a secondary phenomenon. It is also equally possible that humoral and cellular rejection are different and independent processes.

The high incidence of arterial abnormalities in the livers is of special interest in view of the growing evidence that an ischemic vascular factor is of importance in the rejection of skin,[6] cardiac[32] and kidney[15,31,43] homografts. The significance of these changes in hepatic transplants is not known. Moore[24] in postmortem studies has demonstrated areas of poor filling in the hepatic arterial tree, and has suggested that similar devascularization may occur during life. Such changes could not be demonstrated in the present study with *in vivo* angiography, but the degree of small vessel definition was not so great as with his technic. Although acute vascular disease may contribute to the homograft failure, Popper's[30] observations which were cited above as well as the frequent observation in the present study of selective liver cell injury carry the important implication that the hepatocyte rather than the vascular or duct systems may be the primary target.

The auxiliary homograft preparation of Welch and Goodrich and their associates[9,47] proved to be a useful tool in studying many aspects of hepatic homotransplantation. Delineation of the homograft vascularity could be conveniently accomplished. Consistent chronic survivals could be kept for late histologic examination. The selective preservation of the duct system was most evident in this group of animals. The hypertrophy and hyperplasia of the intrahepatic ducts, changes described by Mackay[18] and Paronetto[20] in liver diseases suggested to have an autoimmune basis, were most evident in this preparation.

Nevertheless, the relative ineffectiveness of immunosuppression in the dogs with auxiliary livers is both noteworthy and discouraging in regard to clinical application of this technic. The degree of cellular invasion and hepatocyte loss was much greater than with the orthotopic livers. Furthermore, a very rapid diminution in size occurred after two weeks.

Several possible explanations may account for these findings. Immunosuppressive therapy, which is partly dependent upon monitoring of function, was not delivered so incisively. The abnormal revascularization may have contributed although this is unlikely since dogs with portacaval transposition do not have loss of hepatic mass.[4] Competition with the dog's own liver for nutritional substrate may have been an unfavorable condition. Finally, the dog's own liver with its large reticuloendothelial mass may have contributed substantially to the immunologic reaction in the auxiliary homograft series, a factor which would be eliminated in the orthotopic preparation.

TABLE 3. *Clinical Liver Homografts*

No.	Survival	General Architecture	Reticulum	Intrahepatic Hepatic Artery	Intrahepatic Bile Ducts	Perivenular Necrosis Central Vein	Immunocytes	Kupffer Cells	Hepatocytes
Case 1	4 Hr.	Diffuse autolysis	Intact						
Case 2	22 Da.	Excellent	Intact	Normal	Columnarization and hyperplasia, bile stasis, moderate	None	Periportal, minimal MGP negative	Normal; moderate iron content and small amount bile	Well preserved PAS+
Case 3	7½ Da.	Good; central and scattered focal necrosis, moderate	Intact	Intimal thickening and proliferation	Normal	Moderate	Periportal, moderate MGP negative	Normal	Well preserved PAS +Diffuse fatty metamorphosis
Case 4	6½ Da.	Good; central and scattered focal necrosis, minimal	Intact	Medial and subintimal thickening and focal necrosis	Normal	Slight	Periportal, slight MGP negative	Normal	Well preserved PAS +Intracellular bile pigment
Case 5	23 Da.	Fair; central necrosis, moderate; midzonal necrosis, moderate	Focal areas of linear compression	Medial and intimal thickening, minimal	Normal; bile stasis, moderate	Moderate	Periportal, minimal MGP negative	Normal; moderate iron content	Poorly preserved PAS−; Contain iron and bile pigment, large amounts

Summary

The influence of immunosuppressive drugs upon rejection was evaluated in 40 dogs which received orthotopic or auxiliary hepatic homografts, and in five clinical cases of orthotopic liver transplantation. The effectiveness of azathioprine and steroid therapy was judged upon serial measurements of function, upon duration of survival, and upon findings at pathologic examination.

Definite mitigation of rejection was demonstrated in all three groups. From a histologic point of view, immunosuppression was most successful in the human cases despite the employment of badly ischemiatized cadaveric organs. Orthotopic canine homografts were less completely protected, presumably because of a species difference in the vigor of rejection. The most severely damaged specimens were the canine auxiliary livers which were placed in the right paravertebral gutter without removal of the recipient's own liver.

In dogs treated with immunosuppressive agents, rejection was often observed in the absence of mononuclear cell invasion. The homografts in such dogs commonly had central necrosis and diffuse vascular lesions, with selective preservation of the duct system, the general location of principal hepatocyte loss being the same as previously reported in non-treated animals. The pathophysiologic mechanisms in such non-cellular rejection are considered. It seems possible that the hepatocyte is the primary target of attack by recipient antibodies. Alternatively, the vascular lesions may cause secondary ischemic injury to the parenchyma, in spite of the fact that angiograms in this study failed to support this possibility.

In dogs, the use of optimally preserved homografts makes possible the accurate identification of rejection with biochemical measurements. This diagnosis was made difficult in the clinical cases because of the employement of cadaveric organs, which do not function normally as a consequence of agonal and postmortem ischemic injury. Nevertheless, the results of serial liver tests suggested that rejection was not functionally present in any but Case 5 of the human series during survival periods of 6.5 to 23 days.

Several previously unrecorded observations are described concerning more esoteric biochemical changes after hepatic homotransplantation including alterations in serum or plasma immunoglobulins, amino acids, pyruvates and lactates. In addition, a case is documented in which the haptoglobin genotype of the recipient converted to that of the donor, a finding which supports the concept that the liver is the only source of this substance.

TABLE 4. *Serial Determinations of Serum Immunoglobulin in Patient 5*

Day	$Gamma_2$ mg. %	$Gamma_{1A}$ mg. %	$Gamma_{1M}$ mg. %
−4	860	684	288
−3	860	646	288
+2	640	380	224
4	780	570	512
6	860	418	512
10	740	494	352
12	700	342	416
13	540	372	416
16	600	372	240
19	700	380	416
21	420	289	240
23	420	243	192
Normal Sera			
Mean	1,194	395	191
Range	760–2000	118–1,065	64–380

Addendum

A greatly increased survival after orthotopic canine homotransplantation has been achieved with the combination of daily azathioprine and intermittent intravenous doses of S^{35} methionine which was alluded to in the discussion. Methionine was given every 5 days in a dose of 1.8 mg. carrying approximately 90 microcuries of S^{35}. Eleven of 20 dogs so treated have had survival of more than a month. The longest survival obtained thus far has been 4-1/4 months, the animal still being alive with normal hepatic function on July 28, 1964. Whether or not the improved results are actually due to the addition of radioactive methionine to the regimen or to some other unsuspected factor has yet to be determined.

Since the submission of this manuscript, Dr. Thomas Marchioro has produced strong evidence that competition for alimentary nutritional substrate occurs with the use of the auxiliary liver and that this is the cause for the remarkable diminution of homograft size described in the body of the paper. When the homograft rather than the dog's own liver is vascularized with splanchnic venous flow, the auxiliary homograft retains its size and

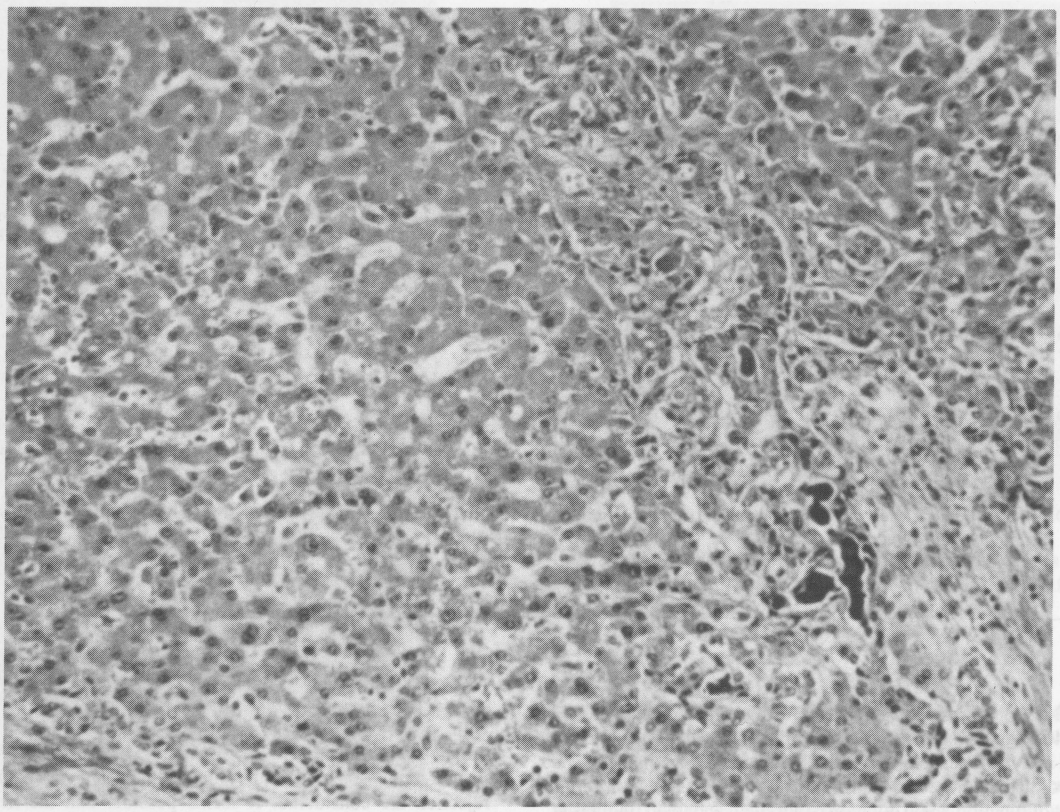

Fig. 17.— Liver homograft from Patient 2 after 21 days. The hepatocytes were well preserved. Note the bile duct hyperplasia (left of field) and the bile accumulation. H and E (from X 80).

selective shrinkage of the autologous liver occurs. Thus, the situation reported in the present study is reversed. The latter observation is of immediate practical significance since it has done much to clarify the physiologic requirements which must be met for the successful use of auxiliary hepatic homografts.

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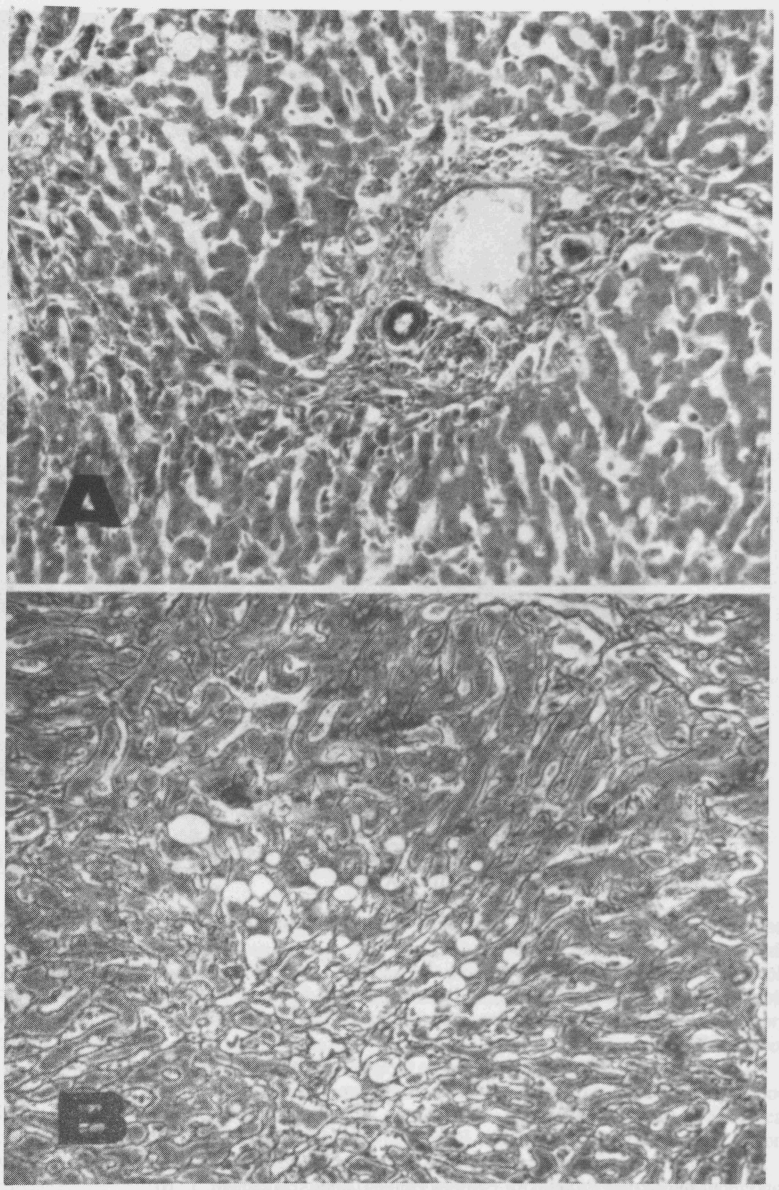

Fig. 18A.— Homograft in Patient 3, after 7.5 days. A. The periportal infiltrate was greater than that seen in the other human cases, but the cells were MGP negative. Ducts are normal. Arterial medial and intimal thickening were seen. PAS stain (from X 80).

Fig. 18B.— Homograft in Patient 3, after 7.5 days. Intact reticular structure. Note fat infiltration, which was proved with Sudan IV stains. Reticulum stain (from X 80).

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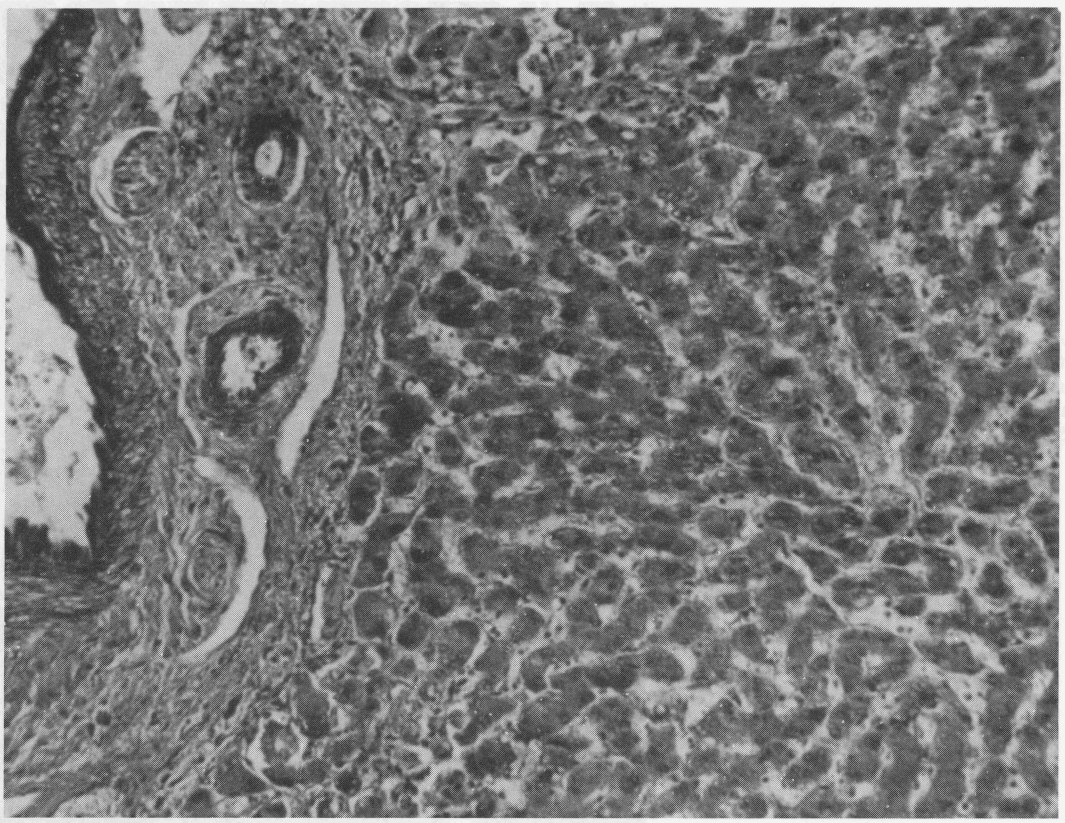

Fig. 19.— Homograft from Patient 4, 6.5 days postoperatively. Liver is almost normal. Note thickening of artery in portal triad. PAS stain (from X 80).

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Discussion

Dr. Francis D. Moore (Boston): I would like to congratulate Dr. Reemtsma and Dr. Starzl for their interesting and very impressive efforts to discern the immunogenetic sequences in heterotransplantation and hepatic rejection.

(Slide) This slide is from a paper that we presented here in 1961, and it shows the healthy liver cells and the immunocytes in the portal area of an untreated hepatic homograft with good liver cell function for many, and a later rejection sequence characteristic of cholangiolitic hepatitis with small bile duct obstruction.

Under immunosuppressive chemotherapy the whole picture is changed. This shows a similar animal at about ten to twelve days. There is practically no immunocytic rejection whatsoever.

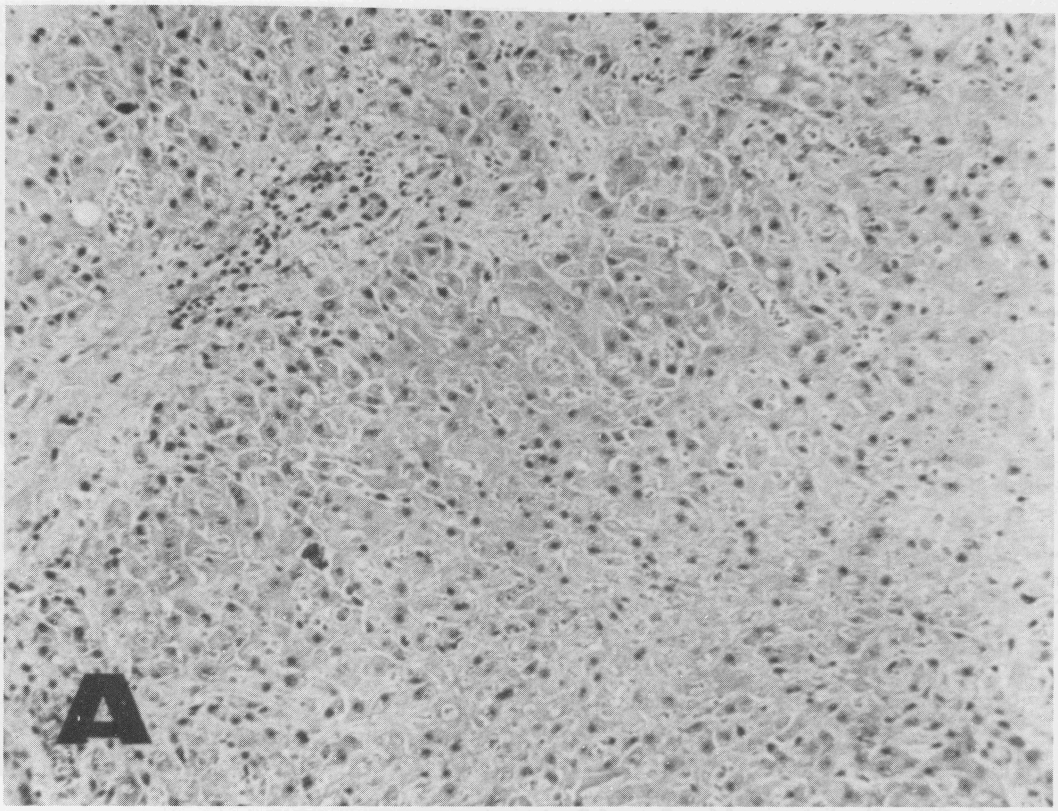

Fig. 20A.— Hepatic homograft from Patient 5. Note the poor staining quality of the liver plates and individual hepatocytes. Immunocytes are rare. H and E stain (from X 80).

(Slide) However, the vascular lesion is most impressive. This is a postmortem hepatic arteriogram of a dog with a hepatic homotransplant under immunosuppressive chemotherapy, and it shows large areas of ischemia with closing off of terminal arterioles which, in serial section, do not show thrombosis.

(Slide) This is an in vivo hepatic homotransplant arteriogram under immunosuppressive chemotherapy. This shows the arterial anastomosis. We can see the dye running into the liver well, but not filling it as well as we would like.

(Slide) And here is the matching portal venogram. Here is the anastomosis here (indicating), filling the liver fairly well but evidently not enough to keep it alive. This is definitely a phase of rejection; it is not seen in the autografts.

While the immunogenetic rejection response is easily abated in liver homotransplantation by the use of immunosuppressive chemotherapy, this has not resulted in the long-term maintenance of canine liver transplants after total hepatectomy. Dr. Starzl has had better results than we have had. We have never had a dog go longer than 14 days. He has had one at 30 days.

In this rapidly growing field of homotransplantation it is important to recognize things that we have not achieved. The long-term laboratory survivor is needed, and we do not have any!

In the case of liver we believe that the cause of this failure is vascular, probably a vasospastic or nonthrombotic vaso-occlusive feature of rejection. Our single human experience corroborated this interpretation.

Such long-term maintenance has likewise not been attained for heart, lung, adrenal, or pancreas, to name but a few organs with which members of this society are laboring.

This point is an important one, because the first successful long-term kidney transplantation in man between individuals related more remotely than fraternal twins was preceded by long-term survival in the laboratory using a protocol very similar, if not identical, to that later used in man.

Intra-order heterografts, such as chimp (or baboon) to man are likewise susceptible to careful laboratory preparation and study. Now that this area has been invaded by assault, it would seem wise to slow down and entrench our position by careful laboratory study of the rejection immunology in intra-order primate heterografts involving the several available primate species *other than man*, in the hope of obtaining laboratory verification before pressing the human patient, *who has something to look forward to from a homograft.*

The ethical problem, in short, is that of science as a whole. Good science is ethical science, and in relation to Dr. Firor's talk, good biological science views the whole man and the whole problem with care and caution.

Dr. C. Stuart Welch (Albany, New York): I rise to add my congratulations to Dr. Starzl and his group for the experimental work which they have been performing, and particularly that which has related to their studies on rejection reversal.

My own interest in transplantation of the liver and in discussing this work is on a technical aspect rather than on the immunosuppression side of it. As Dr. Starzl said, we did our first transplants of the dog's whole liver in the lower abdomen. It is not necessary to place the liver transplants in the usual anatomic site in dogs, even though hepatectomy be done, and it is my belief that when the rejection phenomenon can be shown to be controlled, liver tranplants in the human being will then be justified. At the present time we have not attempted to do any.

I also believe that liver transplants may have their greatest application in treating patients with cirrhosis of the liver, which is a particular interest of mine. In most instances of this diseases hepatectomy need never be done.

Dr. Starzl also suggests here today that from the immunosuppressive aspect it may be important to do it, but if the latter is controlled, hepatectomy should not need to be done in cirrhosis of the liver. At most, perhaps, a portacaval shunt would be necessary, leaving the old liver in place, plus a homotransplant of the liver, which could be done in the lower abdomen. This field of transplantation of the liver should be more rewarding than

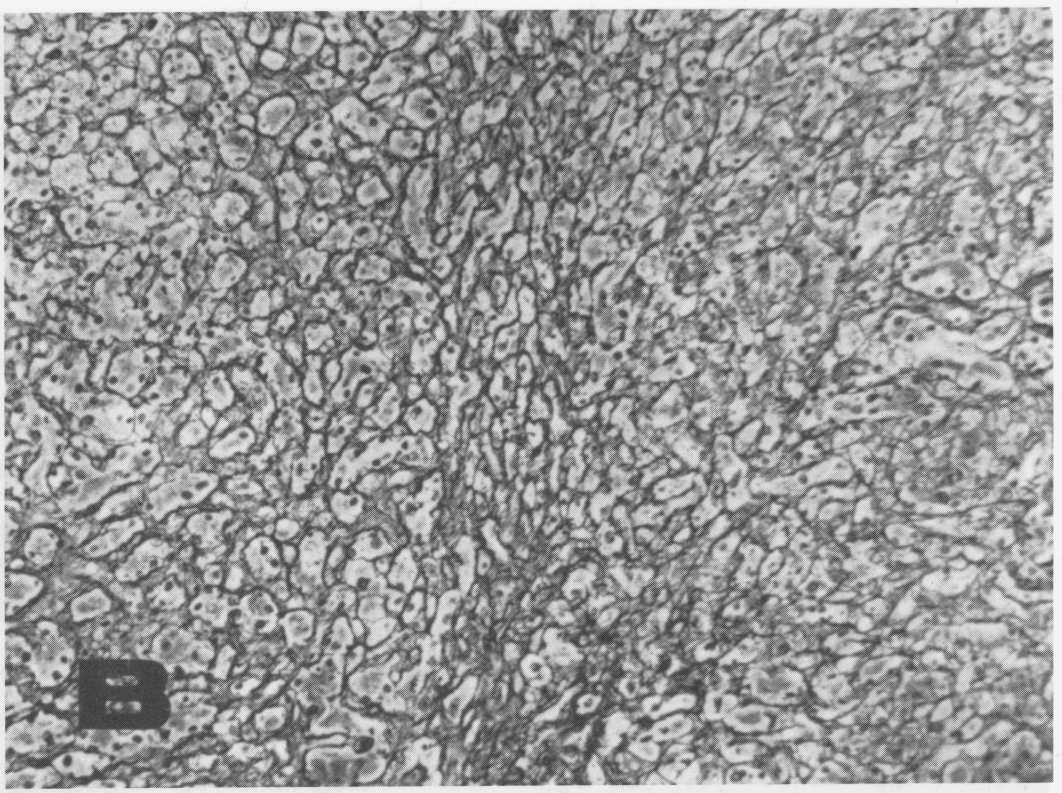

Fig. 20B.— Hepatic homograft from Patient 5. Focal reticular compression, seen in the central part of the field. Reticulum stain (from X 80).

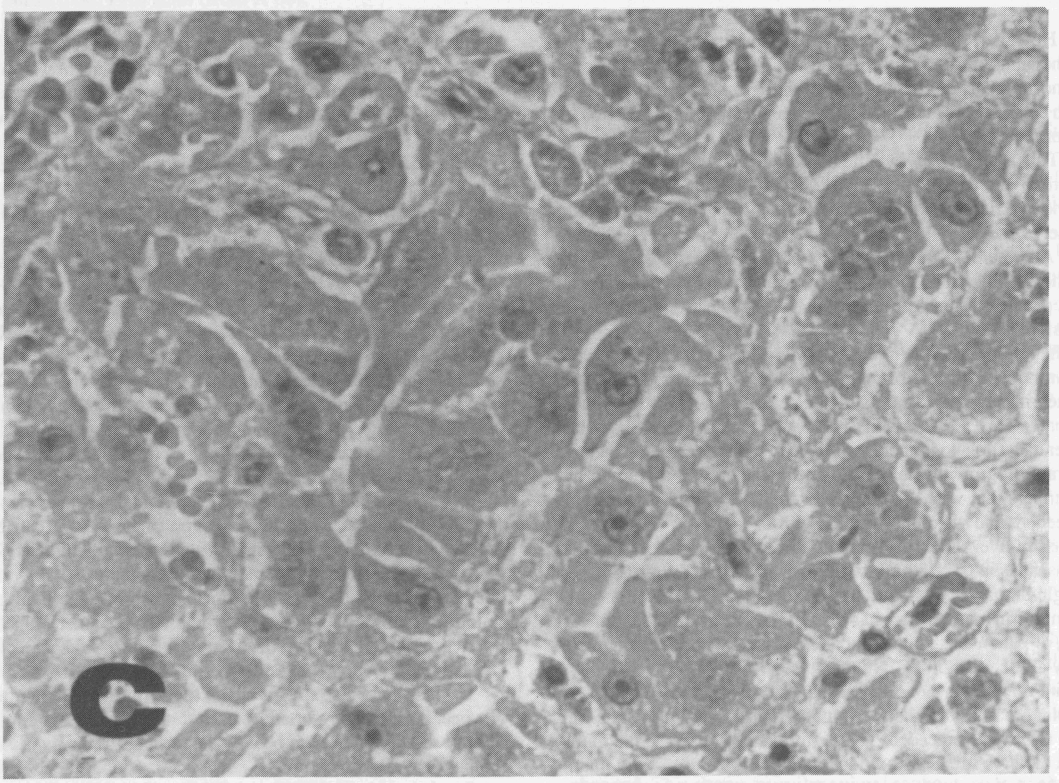

Fig. 20C.— Hepatic homograft from Patient 5. High power view of hepatocytes. Note variation in size and shape of hepatocytes and their nuclei. The liver cells contained no glycogen. PAS stain (from X 320).

hepatectomy and transplantation for cancer of the liver.

The size of the whole liver — and this is one of the technical aspects I wish to discuss — offers some obstacle to lower abdominal transplantation, and we have been working on this subject. In recent experiments we have shown that only half of a liver (homotransplant) will sustain life in hepatectomized dogs and not only do some of these livers live but dogs will live hepactectomized when only the arterial blood supply is reconstituted.

Of 22 experiments of hepatectomy plus homotransplantation of half the liver, six were successful, in that they survived over 24 hours, but four had rather longer survivals. Four homotransplants of the middle and left lobe in hepatectomized dogs survived from four to 12 days. These are only preliminary experiments, but the suggestion is present that portions of the whole liver may ultimately survive, which means that a source of homotransplants from live sources may be possible.

I believe the transplantation into the lower abdomen is easier to do, and may well be the site of choice for transplantation in many diseases, and particularly in cirrhosis, when it becomes feasible to do these on a large scale.

Dr. Joseph E. Murray (Boston): I'd just like to ask one question of Dr. Starzl, and possibly Dr. Moore.

Is it a valid assumption that immune suppression is adequate because cellular infiltrate is lacking? In kidney transplants under immune suppression we see as many as five or six different microscopic patterns of rejection only one of which is characterized by cellular infiltrate. Dr. Moore has intimated that the vascular lesion which he described is on an immunological basis and I wonder if the liver program might better be directed toward testing some other drugs unrelated to azathioprine. The vascular lesion which Dr. Starzl and Dr. Moore describes almost certainly has an immunologic component to it.

Dr. T. E. Starzl (closing): In answer to Dr. Murray's comments, it has not been our assumption that the absence of immunocytes means that the rejection process has been controlled. On the contrary, it is our view that noncellular rejection is typical of the reaction which we are going to see with increasing frequency in livers, just as has been observed with kidneys.

I think his other comments are very appropriate also. At the present time we are working with S-35-methionine in the laboratory, using this radioactive amino acid as an adjuvant to Imuran. The study is not complete, but the results are encouraging.

Concerning Dr. Welch's comments, I think the reasoning behind his pioneer studies was the same as ours. We had hoped that the patient receiving an organ as functionally complex as the liver would have a better chance to survive if during the rejection crisis there were a functional backstop from the animal's or the patient's own organ which could be left in place. However, the inability to control the rejection under these circumstances has been a discouraging one, and I think it is an ill omen as far as any use of this technic employing auxiliary organs.

The explanation for the less favorable behavior of the auxiliary homograft is not clear. It may be that the injured liver is competing for substrate with the animal's own liver; that we are not tracking liver function so well, and therefore not treating rejection as effectively; that diversion of the portal flow and venous revascularization from the systemic system might be a factor, although Dr. Child's studies of ten years or more ago with transposition are against this; or that by removal of the animal's own liver there is a loss of the reticuloendothelial mass in one versus the other situation.

Dr. Moore's philosophic comments are certainly appropriate and, as he knows, they express our opinions also, at least as far as the liver work is concerned.

These experiments were carried out at the University of Colorado in 1963 and 1964 and presented to the Society of University Surgeons in February, 1965. Truly long-term survival was demonstrated repeatedly after transplantation between unrelated mongrel dogs under azathioprine. Ten dogs lived for at least four postoperative months at which time treatment was stopped. Several of the remaining dogs survived for years after stopping all treatment. This led to speculation that the liver was an immunologically privileged organ as originally suggested by Cannon. (cf. Cannon's article, Part I)

Factors determining short- and long-term survival after orthotopic liver homotransplantation in the dog

Surgery, 58: 131-55, 1965

Thomas E. Starzl, Thomas L. Marchioro, Ken A. Porter, Paul D. Taylor, Tanous D. Faris
Thomas J. Herrmann, Charles J. Hlad and William R. Waddell

In an effort to answer many unresolved questions concerning orthotopic homotransplantation of the canine liver, a complete reappraisal of this preparation was undertaken in early 1964 in both the untreated and modified host. A particular effort was made to (A) reduce the operative mortality; (B) interpret the significance of pathologic changes in the homograft and in the recipient tissues; (C) define the presence or absence of a graft-host reaction; (D) study the effect of variations in therapy upon results; (E) assess the hepatotoxic properties of azathioprine; (F) determine if and with what regularity hepatic rejection could be reversed and chronic survival attained; and (G) find if and when a state of relative host-graft nonreactivity developed some time after homotransplantation.

METHODS

Mongrel dogs weighing 8.3 to 27.3 kilograms were used. All animals had hematologic and liver function determinations before and at regular intervals after the experiments were begun. Red cell survival studies were performed with a Cr51 technique.[6] Tetracycline and chloramphenicol were routinely administered. Azathioprine* was employed in most experiments with a dose of 2 to 8 mg. per kilogram per day. Insofar as possible, the induction of leukopenia was avoided. Azathioprine and antibiotic therapy was discontinued at 120 days in all surviving animals. Other variations in therapy are described below. Tissues were examined with light and electron microscopy.

For homotransplantation, livers were obtained from donors of dissimilar appearance, but of approximately the same weight as the recipients. Prior to removal, the donor liver was cooled by perfusion of chilled Ringer's lactate solution through the portal vein. The technique of transplantation[24] resulted in an essentially normal blood supply (Fig. 1). The intervals of ischemia were almost all less than one hour. Cholecystoduodenostomy was established for internal biliary drainage.

* Imuran supplied by Burroughs and Company, Inc., Tuckhoe, N. Y.

Azathioprine toxicity study. In 18 nontransplanted dogs the effects of 40 days of azathioprine were studied. The animals were divided into 3 groups of 6 each which received:

Group I. Daily azathioprine (2 to 4 mg. per kilogram).

Group II. Daily azathioprine plus 1 Gm. intravenous L-methionine.** The methionine powder was dissolved in 100 ml. saline and given in 30 minutes.

Group III. Daily azathioprine plus intravenous S^{35}-methionine every 5 days. Since the specific activity of the radioisotope varied, the amount of L-methionine ranged from 0.08 to 0.6 mg. for the S^{35} dose of 100μc. Based upon the measured biologic half-life (18 to 28 days), and assuming equal distribution of S^{35} to all metabolizing cells, daily total-body irradiation was estimated to begin at 0.2 or 0.3 r per day, with equilibration at 1 r per day after 18 to 28 days.

Homotransplantation without azathioprine. Three groups of animals were studied after orthotopic homotransplantation:

Group A. Supportive operative care only: 7 dogs.

Group B. Daily intravenous L-methionine (1 Gm.): 7 dogs.

Group C. S^{35}-methionine every 5 days: 9 dogs.

Homotransplantation with azathioprine. Azathioprine was begun on the day of transplantation in all dogs except those of Group 5, which received pretreatment for 40 days. Since those animals lost during or shortly after operation invariably died from causes other than failure to control rejection, as many dogs were included in each series (except Group 5) as were necessary to obtain 11 which survived one week or longer. For statistical comparison of the effectiveness of differing regimens, only these selected 11 animals were included. Eight separate series were studied in the following chronology:

** X-ray spectrographic analysis by Dr. Fred Leaver (Denver Veterans Administration Hospital) revealed a selenium concentration of approximately 150 μg per gram methionine.

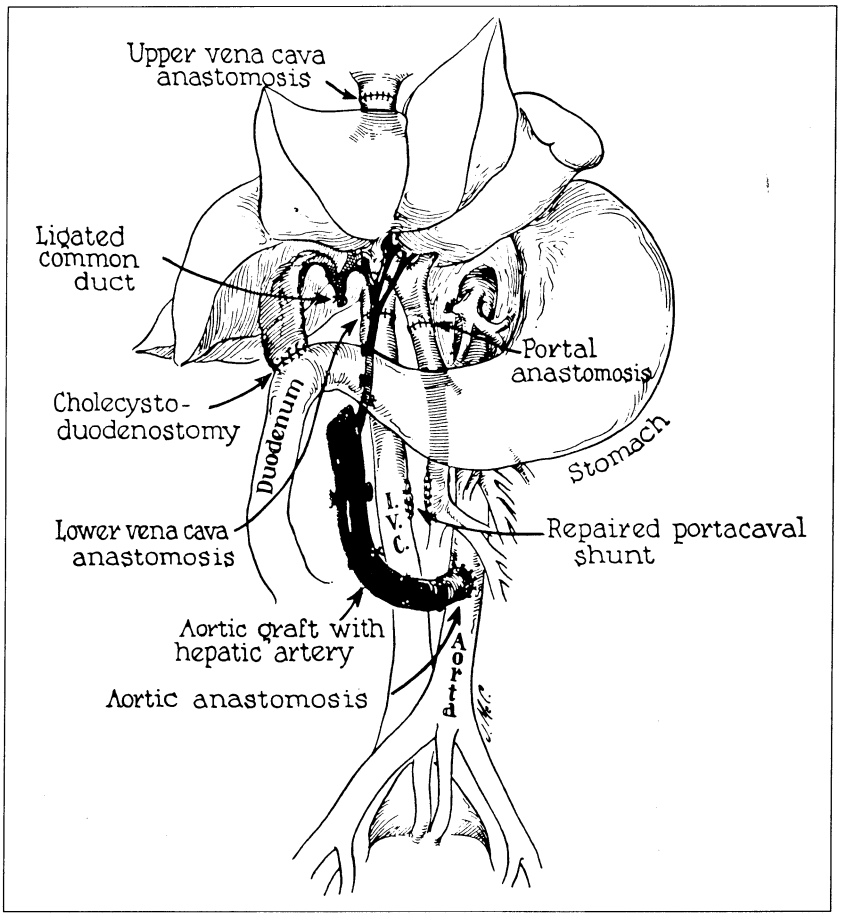

Fig. 1.— Reconstruction after orthotopic liver homotransplantation. Internal biliary drainage is with a cholecystoduodenostomy. Note that the aorta is transplanted in continuity with the hepatic artery of the homograft.

Group 1. Azathioprine, S^{35}-methionine every 5 days, splenectomy: 17 dogs.

Group 2. Azathioprine, S^{35}-methionine every 5 days: 16 dogs.

Group 3. Azathioprine, 1 Gm. per day L-methionine: 13 dogs.

Group 4. Azathioprine only: 16 dogs.

Group 5. Azathioprine pretreatment: 12 dogs which survived the 40 day azathioprine toxicity study and were subsequently provided with a homograft. (Postoperatively, 6 were treated as Group 2; 5 as Group 3; and 1 as Group 4. Only 7 survived a week or more, an early postoperative mortality so great that the study was discontinued.)

Group 6. Azathioprine, 2 mg. methionine every 5 days: 12 dogs. The dose schedule of methionine was comparable to those of Groups 1, 2, and 7, lacking only the radioisotope.

Group 7. Azathioprine, 1 Gm. per day L-methionine, S^{35}-methionine every 5 days, 10 mg. per kilogram choline per day: 17 dogs.

Group 8. Azathioprine only: 13 dogs. This series was treated exactly like Group 4, to determine if unrecognized variations in care had been introduced during the prolonged investigation.

RESULTS

Azathioprine toxicity study.

Mortality and clinical course. Twelve of the 18 nontransplanted dogs lived through the toxicity study, 1 from Group I, 5 from Group II, and 6 from Group III. The other animals died after 18 to 33 days; 3 with bone marrow depression and pneumonitis, 2 from pneumonitis without leukopenia, and 1 of unknown cause. All dogs lost weight during the first three weeks (Fig. 2). Declines in hematocrit were noted in all 18 dogs (Fig. 2), affecting the three experimental groups equally.

Biochemical changes. Although none of the animals became jaundiced, there was evidence of liver injury in all. Rises in SGPT, SGOT, and alkaline phosphatase (Fig. 2). were invariably observed within 2 to 7 days after beginning azathioprine. These changes were severe, usually peaking within 2 weeks and then declining toward normal, despite continuation of therapy. However, return to completely normal values did not occur in any of the dogs. The addition of L-methionine or radioactive methionine did not alter the pattern of injury (Fig. 2).

Pathologic changes in the liver. (Studies done by K.A.P.) Twelve of the 18 livers were abnormal. In all 12, the centrizonal area was most affected (Fig. 3), with either frank necrosis (7 dogs) or pallor of the hepatocytes (5 dogs). Five of the 7 most severely damaged livers had other findings; 5 with bile thrombi in the central and midzonal canaliculi, 2 with intracytoplasmic fat in the midzone, and 2 with scattered focal necrosis. The centrilobular changes were noted in all 6 livers of Group I, 5 of the 6 in Group II, and only 1 of Group III. Mononuclear cell infiltration was not observed.

Four livers were examined electron microscopically, 3 of which had the centrizonal changes note above. In one, which had appeared normal under light microscopy, the hepatocytes around the central veins had a decreased glycogen, a reduction in rough endoplasmic reticulum, and an increase in lipofuscin granules. In the other 3, these changes were more pronounced, extending in one liver to the midzonal area. In 2 of the livers there was dilatation of the bile canaliculi, with thinning and shortening of their microvilli.

Changes in other organs. (Studies done by K. A. P.) Six animals had complete autopsy after 18 to 33 days of azathioprine therapy. Three that died of marrow aplasia had severe depletion of large and small lympho-

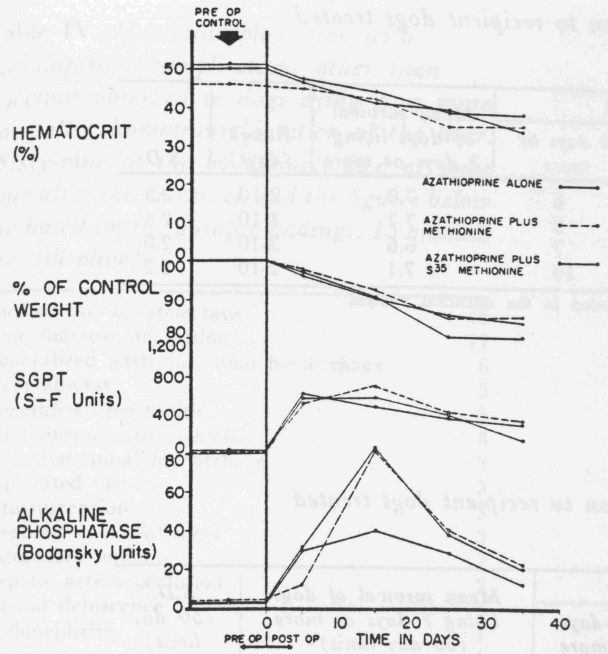

Fig. 2.— Toxicity of azathioprine when used alone, with S-35-methionine, and with L-methionine. Six dogs were in each of the 3 test groups depicted. Despite the abnormalities of liver function, jaundice did not develop.

cytes in the lymphoid tissue. Three others had moderate atrophy and hyperemia of the lymph nodes and spleen, with normal thymus and bone marrow. Pneumonitis and pulmonary congestion were present in 5 animals. Megakaryocytes were often seen lodged in the alveolar capillaries. The other organs were unaffected.

Homotransplantation without azathioprine.

Operative mortality and survival. The only operative death resulted from hemorrhage. The remaining 22 animals survived for at least 2 days and 19 lived for 6 days or longer. All dogs were dead at 10 days. The mean survival was 7.1 ± 2.2 (S.D.) days. Many animals had pathologic processes other than rejection which contributed to death: pneumonitis (12 cases), intussusception (6 cases), and multiple gastrointestinal ulcerations (2 cases). The three subgroups were all comparable, animals receiving no therapy having no difference in survival than those treated with L-methionine or radioactive methionine (Table I).

Clinical course. The clinical course and biochemical alterations were no different than those previously described in the pioneer studies of Moore and co-workers[16] and in our experience.[25] By the fourth day, all animals had marked increases in alkaline phosphatase, SGOT, and SGPT, and by the fifth day, 17 of 19 dogs had hyperbilirubinemia.

Pathologic changes in the liver. (Studies done by K. A. P.) Interstitial edema, prominence of Kupffer cells, and necrosis of a few hepatocytes in areas of centrilobular congestion were present in the first 48 hours, changes which are nonspecific, since they have also been seen in liver autografts.[13] From the third day onward, the histologic features of rejection[1,13,16,25] were present in all but 2 homografts; these consisted of mononuclear cell infiltrations around and within the walls of small portal and central veins, centrilobular and midzonal hepatocyte necrosis, distortion of the centrally located sinusoids (often with mononuclear cells adhering to the endothelium), hypertrophy of bile- or hemosiderin-laden Kupffer cells, and intrahepatic bile stasis in association with destruction of bile duct epithelium. The severity of damage was related to duration of survival, and was not influenced by methionine therapy. In 14 animals only a thin rim of hepatic cells remained around the portal tracts and some of the longest survivors had reticulin collapse and early fibrosis around the central veins. With time, the character of the mononuclear cell infiltration changed. At first, approximately 40 percent of these cells possessed pyroninophilic cytoplasm, but few were typical mature plasma cells. The majority were difficult to classify and were the cells variously called "large lymphoid,"[22] "hemocytoblasts,"[7] and "immunoblasts."[4] After the sixth day the infiltrate was very dense in all the small portal tracts and contained a higher proportion of plasma cells. Fibrinoid necrosis in the walls of small hepatic arteries was seen in only 5 of the 23 dogs.

Changes in other organs. (Studies done by K. A. P.) In the 19 animals dying after the third day the mesenteric lymph nodes were swollen, partly as a result of an intense proliferation of large cells with pyroninophilic cytoplasm. Macrophages containing hemosiderin and debris were present in larger numbers than normal. In those animals dying after the seventh day, the medullary cords were crammed with plasma cells. Similar changes were found in the spleens. Pulmonary congestion, hemorrhage, and bronchopneumonia were common in these animals, while focal thickening of the alveolar septa and interstitial infiltration by mononuclear cells were seen in only a few dogs. Megakaryocytes were found trapped in the alveolar capillaries in all lungs. Myocardial and renal lesions were not seen, and fibrinoid necrotic lesions of arterial walls were not encountered in organs other than the liver.

Homotransplantation with azathioprine.

Operative mortality and survival. Thirty-two of the 116 recipients (27.5 percent) died at operation or during the first postoperative week (Table II), most commonly of pneumonitis (or atelectasis) and intussusception (Table III). These 32 acute failures were considered operative deaths and were eliminated from analysis of the efficacy of immunosuppression.

In the 84 remaining dogs (72.5 percent) which lived for 7 days or longer, there was a high incidence of similar complications (Table IV), but there was usually also biochemical or histologic evidence that some degree of homograft rejection was present. Of the 84 dogs culled for definitive analysis, 44 lived for 25 days and 24 lived for 50 days or longer. Eight more died between days 50 and 120 of causes listed in Table V. Abnormal hepatic function was present in all 8, although other lethal complications were present in each. Another animal which died after discontinuance of azathioprine therapy is discussed below. Fifteen dogs are still alive, from 62 to 324 days after operation (Table V).

Hematologic changes. In virtually every animal a decline in hematocrit was observed, similar to that observed in nontransplant animals receiving azathioprine. The 5 dogs surviving longest started with a mean preoperative hematocrit of 47.5 percent, which had fallen to 36.8 percent by the time azathioprine was discontinued on postoperative day 120. Forty days later the mean hematocrit had returned to 43.5 percent. The increase in hematocrit after discontinuance of azathioprine is strong evidence that

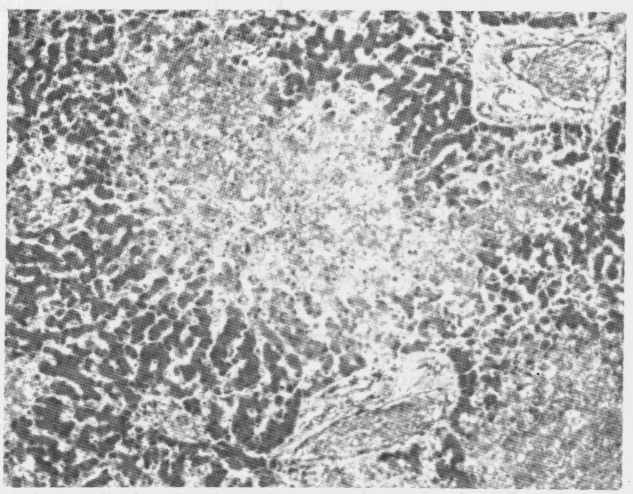

Fig. 3.— Liver of a dog treated with azathioprine for 26 days. There is centrilobular necrosis of hepatocytes but no cellular infiltration. (Hematoxylin and eosin. Original magnification X 40.)

Table I. *Survival after hepatic homotransplantation to recipient dogs treated with azathioprine*

Group	No. dogs	Survival		Mean survival of dogs living 2 days or more	Range (days)	S.D.
		2 days or more	6 days or more			
I (no therapy)	7	7	6	7.0	2-10	2.5
II (methionine)	7	7	6	7.7	3-10	2.4
III (S^{35}-methionine)	9*	8	7	6.6	3-10	2.0
	23	22	19	7.1	2-10	2.2

*One dog did not survive operation and is therefore not included in the statistical analysis

Table II. *Survival after hepatic homotransplantation to recipient dogs treated with azathioprine*

Group	No. dogs	Survival			Mean survival of dogs living 7 days or more (50 day limit)*		S.D. (50 day limit)
		7 days or more	25 days or more	50 days or more			
1†	17	11	8	4	33.4	72.3	16.4
2†	16	11	7	5	31.7	68.8	19.3
3†	13	11	7	3	29.5	63.1	15.0
4†	16	11	4	2	24.2	39.1	14.5
5	12	7	2	1	19.7	28.6	15.6
6	12	11	5	3	26.5	32.2	18.5
7	17	11	7	3	28.9	37.1	15.6
8	13	11	4	3	26.1	30.8	18.1
	116	84	44	24	27.9	47.3	16.5

*The dogs surviving longer than 50 days are described individually in Table V, the greatest longevity now being 324 days. Statistical evaluation of different regimens take into account only the first 50 days. This avoids introduction of unfair bias in the event of unusually protracted survival, and allows comparison of different series of animals undergoing operation in the chronology shown. In all of the groups, the actual mean survival without the artificial 50 day limit has been brought up to date to Feb. 10, 1965, and is just to the right of the artifactually low values.

†In at least one dog from each group, all therapy, including azathioprine, was discontinued after 120 days.

Table III. *Homotransplantation with azathioprine; nonimmunologic conditions which contributed to or were the direct cause of death of 32 animals during operation or in the first postoperative week. Several animals had more than one complication*

Pneumonitis or atelectasis	13
Intussusception	9
Hemorrhagic gastroenteritis	5
Hepatic arterial occlusion	5
Pulmonary emboli	2
Operative hemorrhage	2
Portal vein occlusion	1
Total	37

this agent was primarily responsible for the chronic anemia, presumably from inhibition of erythropoiesis.

In addition, a contributory early factor seemed to be increased red cell destruction, which may have been due to erythroplastic activity of the liver,[9] in view of the prominent hemosiderin deposits (to be described subsequently) in many of the homografts. Seven animals studied from 7 to 27 days after homotransplantation had a red cell half-life of 6.7 ± 3 (S.D.) days (normal for this laboratory, 14 days). After the first month, the half-life gradually returned toward normal (Fig. 4).

Clinical course, biochemical changes, and reversal of rejection. The course after homotransplantation was extremely variable. In 24 (28.6 percent) of the 84 definitive test animals rejection was uncontrollable. Inexorable jaundice developed (Fig. 5), with anorexia and rapid weight loss. Rises in SGOT, SGPT, and alkaline phosphatase (Fig. 5) were similar to, but of generally greater magnitude, than those produced by azathioprine administration to the nontransplanted dog. Terminal convulsions or coma, intractable vomiting, and hypoglycemia were common. All 24 animals died in less than 41 days, with a mean survival of only 15 days.

A more benign rejection was observed in 41 of 84 experiments (48.3 percent) despite the fact that hyperbilirubinemia and rises in SGOT, SGPT, and alkaline phsophatase were also present (Figs. 6 and 7), sometimes to the same degree as in the 24 animals noted above. However, the biochemical indices of hepatic injury were partially (Fig. 7), or even relatively completely, reversible (Fig. 6). At the peak of rejection, which lasted as long as 6 weeks in some cases, these dogs often appeared to be in terminal

Table IV. *Homotransplantation with azathioprine; complications other than rejection observed in dogs dying 7 or more days after homotransplantation of the liver. (Sixty-nine of the 84 animals died at some time after the first week and the figures below are based on the autopsy findings; 15 animals are still alive)*

Pneumonitis or atelectasis	46
Bone marrow depression	11
Generalized gastrointestinal hemorrhage	6
Liver abscess	3
Generalized peritonitis	3
Subdiaphragmatic abscess	3
Intra-abdominal hemorrhage	3
Perforated viscus	3
Intussusception	3
Bleeding duodenal ulcer	3
Pulmonary emboli	2
Hepatic artery occlusion	2
Wound dehiscence	1
Pyelonephritis	1
Leg gangrene	1
Thermal burn of back	1
Total	92

condition, with clay-colored stools, dark urine, profound anorexia, and rapid weight loss. As liver chemistries subsequently improved, most of the animals had a return of appetite. A number of the dogs ultimately died from complications of chronic liver insufficiency, such as gastrointestinal hemorrhage, or from pneumonitis, but others continued to live for many weeks or months with gradually improving liver function. The longest survival of any animal with this type of reversible rejection was 161 days (Fig. 7). Sixteen others lived for 50 days or more, indicating that protracted survival under these unfavorable circumstances was not rare.

In the other 19 dogs (22.6 percent), overt rejection did not occur. Although there were rises in alkaline phosphatase, SGOT, and SGPT, the serum bilirubin did not exceed 2.5 mg. percent at any time during postoperative azathioprine treatment (Fig. 8). Weight loss and anorexia were not pronounced. Seven dogs died from other causes than rejection before 25 days, so that failure to develop jaundice may have been more a function of the short survival than anything else. The other 12 all lived for more than 25 days. Five died after 28 to 35 days without developing hyperbilirubinemia, and the other 7 are still alive after 90, 116, 201, 218, 228, and 324 days (Table V). Five dogs from this favored group had azathioprine discontinued at 116 to 123 days as described below.

Chronic liver function. The last routine liver chemistry studies done an all dogs living beyond 50 days are shown in Table V. In 6 of these animals other determinations were performed from 98 to 300 days postoperatively. Bromsulfonphthalein retention at 45 minutes was 5 percent or less in 4, 8.2 percent in 1, and 34 percent in 1. Prothrombin times were 52 to 90 percent. Total proteins were 4.8 to 5.8 Gm. percent.

Host-graft nonreactivity. Five nonjaundiced animals had all therapy discontinued after 116-123 days. All are still alive 63, 81, 100, 109, and 204 days later. One had a delayed rejection, beginning 2 weeks after stopping azathioprine, but the hyperbilirubinemia and enzyme increases ultimately stabilized and reversed without treatment (Fig. 9). The other 4 have had no deterioration of homograft function in the ensuing 81 to 204 days (Fig. 8). In all cases, irregular increases in the peripheral leukocyte count were observed during the first 2 months after cessation of therapy.

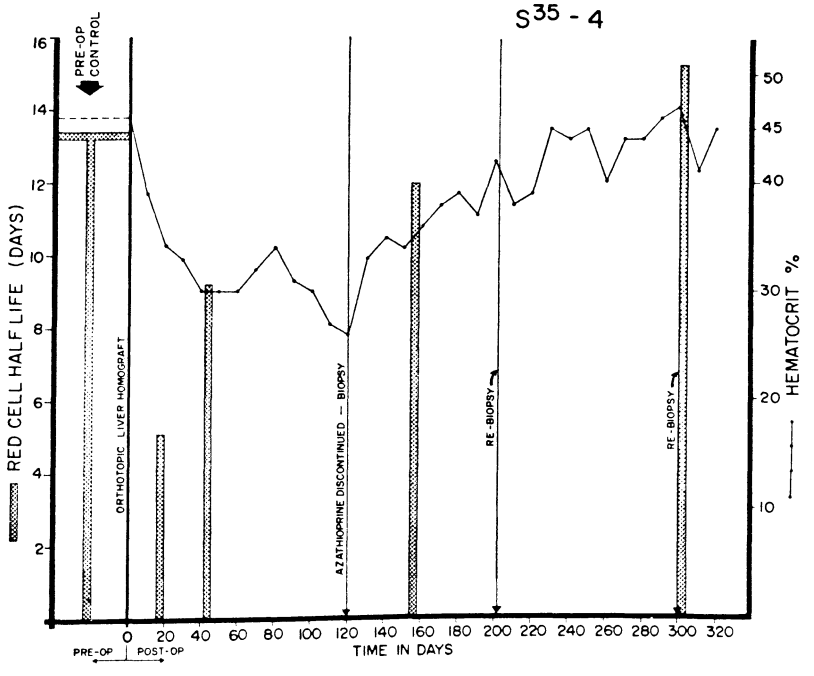

Fig. 4.— Red cell survival and hematocrit values in a dog that is still alive 324 days after orthotopic transplantation. Note the sharp reduction in red cell half-life in the first postoperative month, with gradual return toward normal. Red cell survival was not altered by withdrawal of azathioprine at the end of 4 months, but the depressed hematocrit rose sharply during the succeeding months.

Table V. *Homotransplantation with immunosuppression (fate of 24 animals living 50 days or longer; the survival figures for dogs still living is brought up to Feb. 10, 1965)*

Group	No. dogs	Survival (days)	Gross cause of death	Last bilirubin (mg. %)	Last alkaline phosphatase (B.U.*)	Last SGOT (S-F.U.†)
1	1	324	Alive‡	0.1	1.9	23
	2	98	Pneumonitis; liver failure	5.7	503	760
	3	116	Alive	0.3	58	62
	4	90	Alive	0.3	172	94
2	1	120	Gastrointestinal hemorrhage; liver failure	3.7	296	870
	2	58	Pneumonitis	1.3	300	220
	3	228	Alive‡	0.1	73	470
	4	70	Gastrointestinal hemorrhage; liver failure	5.9	516	1660
	5	181	Alive‡	4.1	361	1080
3	1	218	Alive‡	0.1	30	400
	2	101	Pneumonitis; liver failure	5.6	436	580
	3	201	Alive‡	0.1	16	650
4	1	161	Bleeding duodenal ulcer; liver failure‡	4.7	166	110
	2	103	Post biopsy hemorrhage; liver failure	5.7	746	380
5	1	112	Alive	6.2	692	1000
6	1	69	Pneumonitis; liver failure; gastrointestinal hemorrhage	3.9	657	1200
	2	50	Pneumonitis; liver failure	4.8	354	250
	3	93	Alive	3.9	268	1068
7	1	82	Alive	2.8	527	1900
	2	81	Alive	0.2	188	360
	3	77	Alive	6.1	184	500
8	1	72	Alive	4.3	530	1100
	2	68	Alive	4.4	140	37
	3	62	Alive	7.0	473	1500

*Bodansky units.
†Sigma-Frankel units.
‡Azathioprine stopped after 116 to 123 days; subsequent course without immunosuppression.

A sixth animal had serious liver malfunction while under azathioprine therapy (Fig. 7), a maximum bilirubin of 10 mg. percent being present on postoperative day 90. By day 120 this was decreasing and continued to decline after azathioprine therapy was stopped. The animal ultimately died of a bleeding duodenal ulcer 161 days posttransplant.

Effect of adjuvant therapy. Mean survival of the various test groups is shown in Table II. For analytic purposes, no animal was credited with more than 50 days. Within this arbitrary time limit, none of the regimens had a statistically significant superiority. In Groups 1, 2, 3, and 7 in which either S^{35}-methionine and/or 1 Gm. per day L-methionine were administered, mean survival exceeded that of Groups 4 and 8, in which these agents were not used, and was greater than Group 6, in which 2 mg. nonradioactive methionine was given every 5 days. However, even comparison of the series combinations (Groups 1, 2, 3, 7, versus 4, 6, 8; or 1, 2, 3, 7 versus 4, 8) did not show a statistically significant advantage of the adjuvant therapy ($p = 0.18$ and 0.17).

Group 5, which differed from the other 7 series in that 40 days of azathioprine pretreatment was employed, had the shortest mean survival. This seemed attributable to a heightened incidence of infection, a septic complication being the cause of death in 9 of the 11 failures. Thus, azathioprine pretreatment had an adverse effect after hepatic homotransplantation in contrast to an apparent benefit previously described with renal homografts.[27]

Pathologic changes after homotransplantation with azathioprine (Studies done by K.A.P.)

Homografts in animals dying within one week. Twenty-seven livers were examined and showed the central venular and sinusoidal congestion, variable centrizonal necrosis, interstitial edema, and Kupffer cell swelling already described in the untreated dogs dying in the first 2 postoperative days. In addition, centrilobular bile stasis was usual. Portal and central cellular infiltration was appreciable in only 7 and 2 of the homografts, respectively, consisting of mixed neutrophilic and mononuclear cells, with pyroninophilic cytoplasm being rare in the latter. In several specimens, the perivenular lymphatics were dilated.

Electron microscopy examination of a homograft at 6 days showed a few lymphoid cells around the small portal veins and adhering to the sinusoidal endothelium. Some of these had abundant free cytoplasmic ribosomes arranged in rosettes. The hepatocytes adjacent to the central vein were necrotic; those cells just peripheral to this zone contained many fat droplets and lipofuscin granules, lacked glycogen and rough ergastoplasm, and contained swollen mitochondria. Hepatocytes in the middle and peripheral zones were normal.

Homografts in animals dying in 7 to 15 days. Twenty-seven

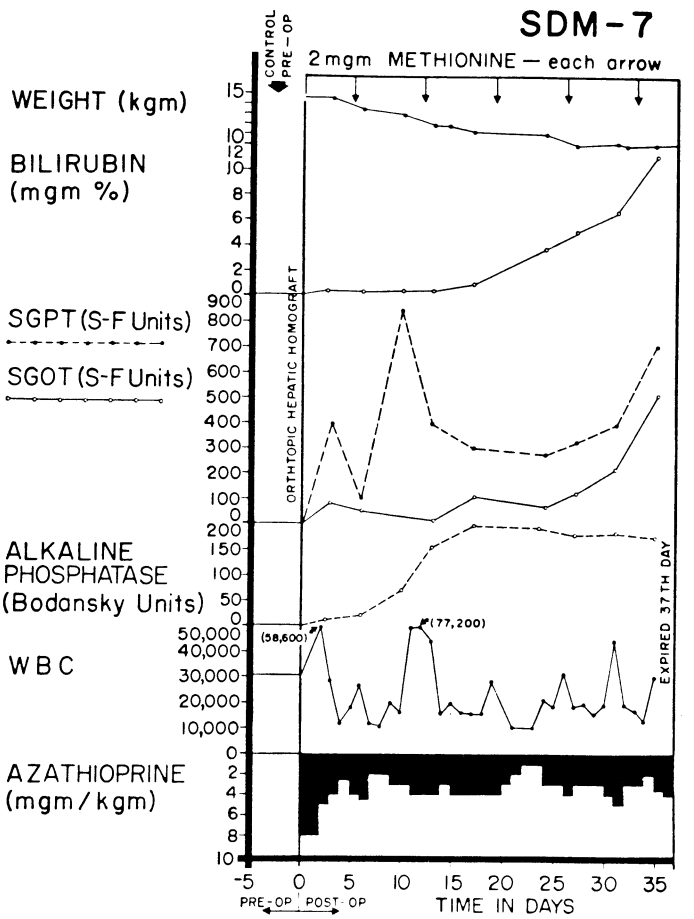

Fig. 5.— An example of inexorable rejection despite immunosuppressive therapy. Determination of the serum bilirubin was the most useful measurement for following the course after homotransplantation since the other abnormalities of liver function depicted can also be caused by azathioprine. Group 6 series.

homografts were examined; central necrosis was present in 22, had extended to the midzone in 17 (Fig. 10), and was often associated with mild to moderate central congestion or sinusoidal distortion. With severe hepatocyte loss, central reticulin collapse had occurred, and in 5 the condensed reticulin bands connected adjacent central veins. Interstitial edema, cholestasis, destruction of bile canalicular epithelium, and bile- or hemosiderin-containing Kupffer cells were also present. In every liver the small, and often the large, portal tracts were moderately (8 cases) or extensively (19 cases) infiltrated by mononuclear cells (Fig. 10), 30 to 40 percent of which possessed pyroninophilic cytoplasm. As in the untreated animals, many were the primitive "large lymphoid" cells; others resembled plasma cells. A similar, but lesser infiltrate was noted in and around the walls of the central veins in 17 instances.

Preliminary ultrastructural analysis of 3 homografts at 8 to 10 days revealed an extension of the findings noted above in the 6-day specimen. The lymphoid cells in the portal tracts and sinusoids were now joined by plasma cells and their precursors with abundant rough endoplasmic reticulum, and the sinusoidal endothelium had disintegrated in many places. Dilatation and destruction of bile canaliculi with microvillar loss and obstruction by masses of bile pigment were common. Many intact hepatocytes exhibited changes in the cytoplasmic organelles as well as in the nuclei.

Homografts in animals dying in 16 to 25 days. In 14 of the 17 livers, mononuclear infiltration around the portal and central veins was observed, but in all but 3 the cells were fewer than had been present after 7 to 15 days and had a decreased percentage with pyroninophilic cytoplasm. The majority were small lymphocytes and plasma cells with some hemosiderin-laden macrophages. Centrilobular hepatocyte loss was seen in 15 homografts but it appeared to be recent only in the 3 specimens with heavy cellular infiltration. The surviving liver cells around necrotic areas often contained fat droplets, bile pigment particles, and excess lipofuscin. Centrizonal reticulin collapse and deposition in these areas of collagen fibers was common, and in 3 livers, bands of connective tissue had linked adjacent central veins. Periportal connective tissue was not increased. Centrilobular bile stasis and hemosiderin deposition were prominent. In one homograft there was focal fibrinoid necrosis of the walls of small hepatic artery branches. (This was the only example of that change in the 101 azathioprine-treated hepatic homografts examined microscopically.)

Three homografts were examined electronmicroscopically at 16, 17, and 25 days. Two of these, which happened to come from the atypical specimens with heavy cellular infiltration, were similar to those in the 7 to 15 day group. The third had only a few numbers of infiltrating cells which were mostly lymphocytes and mature plasma cells. The other findings also confirmed those observed with light microscopy, and revealed that many centrilobular canaliculi had microvillar loss and were plugged with bile pigment. Fat-laden hepatocytes adjacent to areas of necrosis lacked rough ergastoplasm.

Homografts in animals dying in 26 to 50 days. Four of the 18 livers

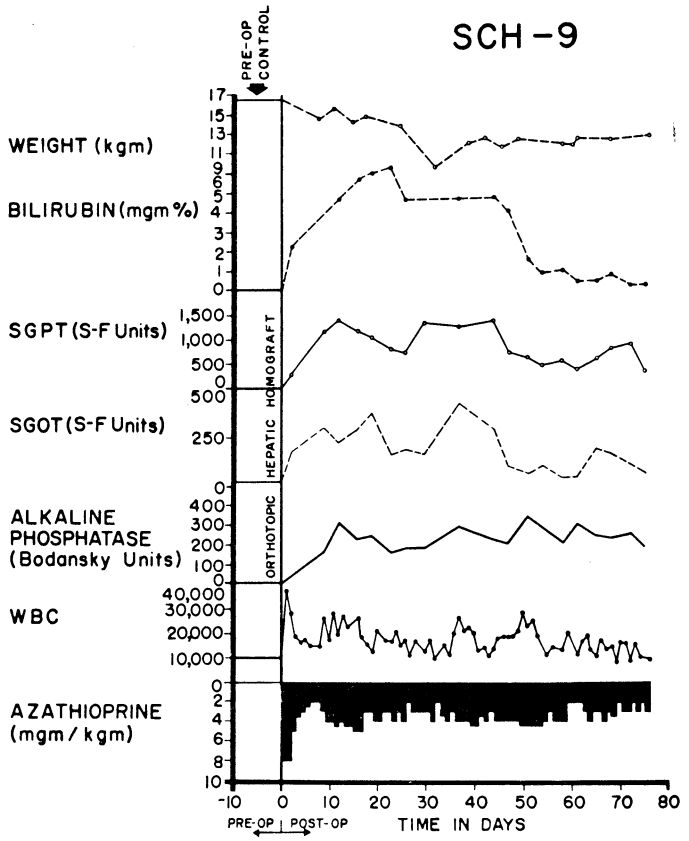

Fig. 6.— Virtually complete reversal of severe hepatic rejection. Note the severe jaundice which ultimately completely disappeared. The animal is still alive. Reversal was accomplished wihout any alteration in immunosuppressive therapy. The animal received adjuvant S-35-methionine, L-methionine, and choline (Group 7).

were completely free of invasion, and in the others the quantity and character of the cellular infiltrate was comparable to that of the previous group. The centrilobular hepatocyte loss was also similar, being present in 15 homografts, but with recent necrosis in only 4. The central reticulin condensation was present in 17, and in 7, connective tissue bands had linked the central veins (Fig. 11). The portal reticulin and connective tissue were also increased in 4 livers, and in one of these there was proliferation of small bile ductules. One homograft had small regeneration nodules lacking normal lobular architecture. The intrahepatic cholestasis and hemosiderin deposition described in the earlier specimens were usually present.

Homografts in animals dying in 51 to 120 days. Infiltration around the portal and central veins was mild in 4 of the homografts and marked in the 3 others. In the former, the cells were almost all lymphocytes or plasma cells, but in the latter, many primitive cells with abundant free cytoplasmic ribosomes were also present. Nonacute central hepatocyte loss with associated reticulin collapse and collagen deposition were similar to that described in the two preceding sections. In 6 of these, connective tissue linked in the central vein, and in 3, several central veins were occluded with fibrous tissue. Portal fibrosis had occurred in all 7 homografts, and some fibrous and reticulin bands connected these areas to the central veins. Regeneration nodules were present in 2 homografts, and in all livers peripheral hepatocytes showed increased basophilia, occasional mitoses, and polyploidy. Centrilobular bile stasis and Kupffer cell hemosiderosis were conspicuous in all the livers.

Effect of withdrawing therapy upon homograft histology. In 3 dogs the homograft was biopsied at 120 to 123 days when all immunosuppressive drugs were withdrawn. A further biopsy was then obtained 77 to 84 days later. One animal was biopsied for a third time 182 days after withdrawal of therapy. The animal whose liver was sampled on 3 occasions was unique in that all specimens appeared to be within normal limits, both with light (Fig. 12) and electron-microscopy.

A second dog had an abnormal hepatic homograft at the time treatment was discontinued (Fig. 13, A) with moderate infiltration of mixed mononuclear cells (10 percent pyroninophilic) and neutrophiles in the portal areas, and a few mononuclear cells around the central veins. The lobular pattern was distorted with fibrous and reticulin bands which linked portal tracts to each other and occasionally to central veins; by reticulin and collagen condensation around thickened central veins; and by pseudolobules of actively regenerating liver cells. The centrilobular hepatocytes had disappeared and in some areas or were atrophic in others. Many remaining liver cells had decreased basophilia, increased lipofuscin, and pale, foamy cytoplasm; ultrastructurally they contained lipid droplets and lacked glycogen and rough ergastoplasm. In the portal tracts, there was proliferation of small ductules, accumulations of hemosiderin- and lipofuscin-containing macrophages, and occasional nonfibrotic intimal thickening of small hepatic arteries. Bile plugs were not seen. Seventy-seven days later (Fig. 13, B), the cellular infiltration was lighter and lacked neutrophiles, although the percentage of pyronine-positive cells was unchanged. Active proliferation of peripheral liver cells was continuing, but there were fewer regeneration nodules and the hepatocytes appeared to be more normal with both light and electron-microscopy. The fibrotic alterations persisted, but the over-all picture was one of improvement.

A homograft in a third dog had comparable abnormalities at the time therapy was withdrawn (Fig. 14, A) differing only in that fibrosis was somewhat less and regeneration nodules were absent. Eighty-four days

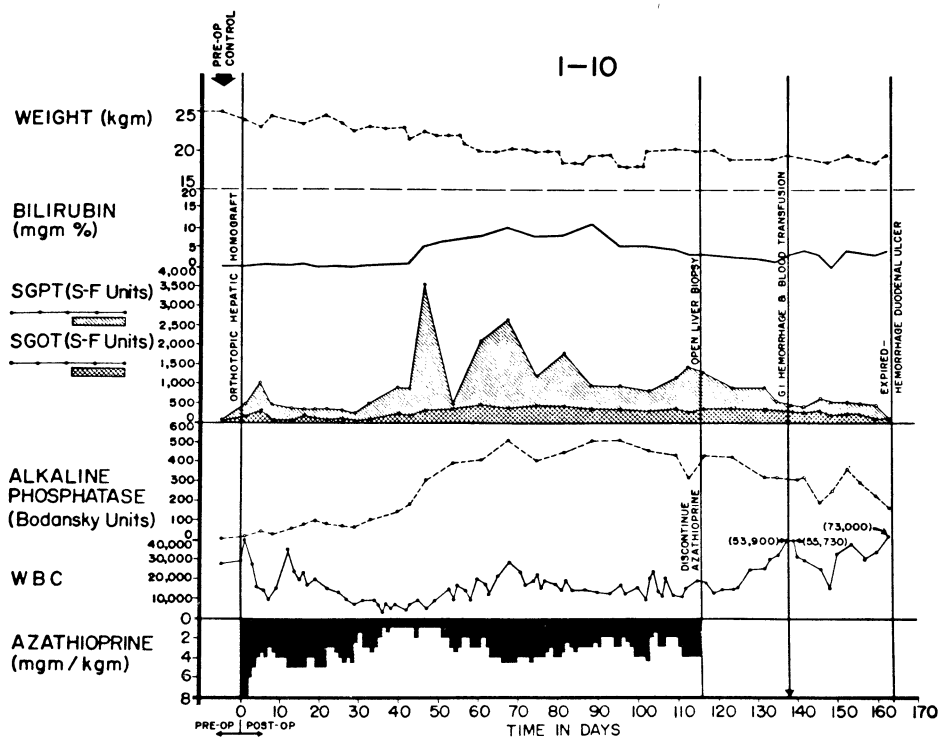

Fig. 7.— Reversal of rejection in an animal treated with azathioprine. Serum bilirubin rose to more than 10 mg. percent and then declined. Note that the improvement in liver chemistries continued after discontinuance of azathioprine therapy at 116 days. Cause of death 45 days later was massive hemorrhage from a large duodenal ulcer. Group 4 series.

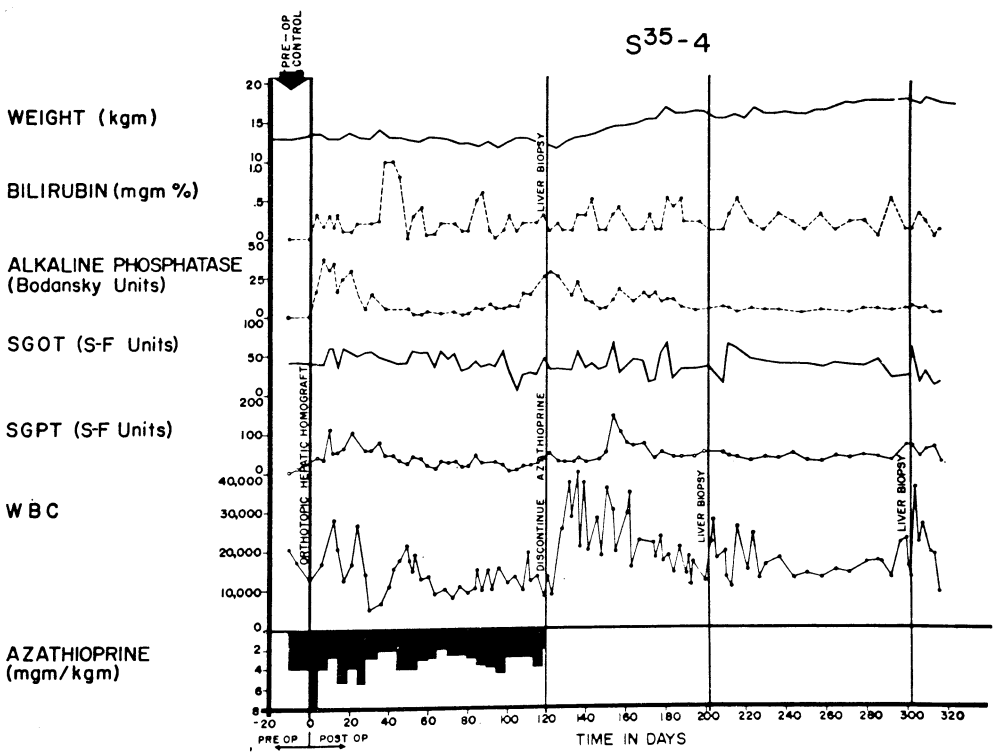

Fig. 8.— Course of an animal which never exhibited any clinical evidence of homograft rejection. Note the rapid weight gain following cessation of therapy at 4 months. The pronounced leukocytosis after withdrawal of immunosuppression was commonly seen. This animal received adjuvant S-35-methionine.

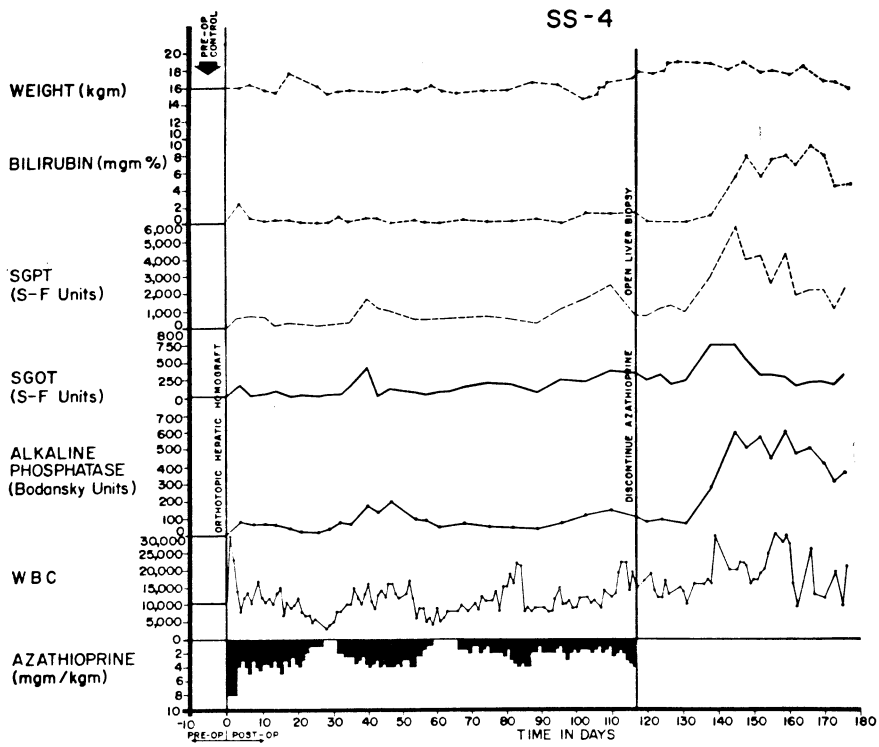

Fig. 9.— Course of an animal which did not have clinically evident rejection during the first 4 postoperative months. After azathioprine was discontinued, there was deterioration of all liver chemistries, despite which the animal appeared healthy and had little weight loss. The late rejection partially reversed without reinstitution of therapy.

later (Fig. 14, B) there was further homograft damage in which many centrally located hepatocytes contained fat droplets. Intrahepatic cholestasis was not present. Cellular infiltration was increased, chiefly due to an increased number of neutrophiles. The deterioration appeared to be due, at least in part, to the effects of cholangitis.

Effect of methionine upon homograft histology. Centrilobular necrosis occurred in 40 percent of animals treated with adjuvant methionine plus azathioprine compared to 64% when azathioprine was used alone. The incidence of cellular infiltration in both groups was 85 percent.

Changes in host tissues in 92 experiments. Changes in the recipient's lymph nodes and spleen roughly paralleled those in the homograft. In 16 experiments in which a heavy infiltrate of pyroninophilic cells was found in the liver, "large lymphoid" and other smaller pyronine-positive cells were found actively proliferating around postcapillary venules in the lymph nodes and around small arteries of the spleen, as well as large numbers of plasma cells in the medullary cords and red pulp. The hyperemic lymph nodes had lost their normal follicular architecture.

In 24 experiments in which moderate numbers of pyroninophilic cells were invading the homograft, the degree of proliferation of similar cells in host lymphoid tissue was variable. Fifty-two homografts had mild or no infiltration of the liver, and here the recipient spleen and lymph nodes resembled those described earlier in animals receiving azathioprine alone, in that "large lymphoid" cells and small lymphocytes were either normal or fewer in number.

Bone marrow changes usually seemed to be related to azathioprine administration. In several animals there was extreme myeloid hypoplasia or even aplasia. There was some evidence of thymic hyperplasia in a few of the dogs that rejected the hepatic homograft after 24 days. The lungs often showed congestion, edema, and intra-alveolar hemorrhage. Bronchopneumonia was a frequent complication. Alveolar wall thickening and sequestration of megakaryocytes were seen. A few of the kidneys showed either small areas of chronic pyelonephritis or scars suggestive of past infection with canine leptospirosis. Necrotic changes in the walls of arteries and arterioles in organs other than the liver were very rare.

DISCUSSION

The present canine studies provide increased hope that orthotopic hepatic homotransplantation may ultimately have use in the treatment of human liver disease. The overwhelming early mortality previously encountered in dogs[14-16,25,26,28] has been substantially reduced. Many long-term survivors were obtained, proving that control of hepatic rejection is not uniquely difficult, and that results can be achieved which are similar to those previously reported for the kidney[2,3]

Instead, the past discouraging experiences seem ascribable to other factors which play an extremely prominent cumulative role. Technical failures are common until considerable experience is acquired. Hemorrhagic gastroenteritis, acute peptic ulceration, and intussusception are frequent. The susceptibility to pneumonitis appeared to be accentuated by the subdiaphragmatic presence of the large homograft. Finally, the hepatotoxicity of azathioprine has a special significance, since the target tissue of rejection is also injured by the drug used to prevent this process.

Because elevations in SGOT, SGPT, and alkaline phosphatase are caused both by azathioprine and by rejection, the serum bilirubin was the most useful test with which to serially study homograft function in the treated canine host. The regularity with which reversal of hepatic rejection could be documented by this means is noteworthy, examples being seen in half of the technically successful experiments. Of great interest is the fact that reversal was not dependent upon intensification of immunosuppressive therapy, since neither increased doses of azathioprine nor additional drugs were used, and in one instance the phenomenon was noted in the absence of all therapy (Fig. 9). It seems probable that rejection of any tissue is characterized by a tendency to remission. Although prednisone,[8,12,29] and less certainly actinomycin C[2] or local irradiation,[10] may be of life-saving value in promoting the process after renal homotransplantation, the use of

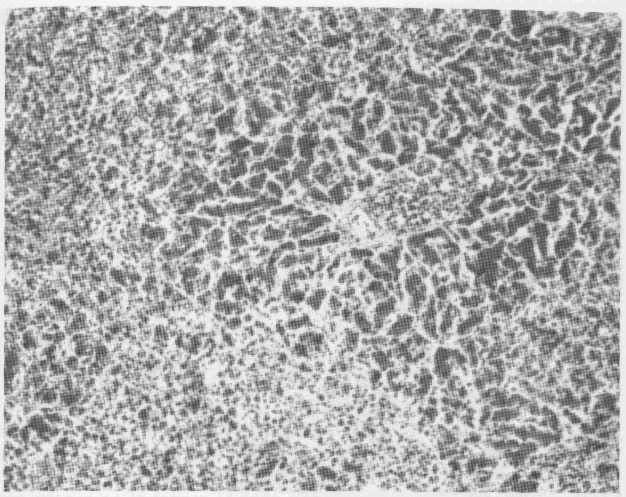

Fig. 10.— Hepatic homograft which has been rejected by 15 days despite azathioprine treatment. There is widespread destruction of hepatocytes in the central and middle zones of the lobules. Only a rim of liver cells remain around the small portal tract, which is heavily infiltrated with mononuclear cells. (Hematoxylin and eosin. Original magnification x 40.)

such additional measures is probably not requisite in some instances regardless of the type of homograft used. The spontaneity with which reversal may occur makes caution necessary in attributing this effect to any preceding variation in therapy.

A related problem in evaluating treatment in mongrel animals or man is the extreme variability of host response to the foreign tissue. In the present study, this ranged from complete absence of rejection to uncontrollable repudiation of the liver homograft, a spectrum which undoubtedly conformed to the degree of chance antigen matching between donors and recipients. The presence in outbred populations of this powerful and unpredictable influence makes conclusions about minor therapeutic alterations meaningless unless hundreds or even thousands of experiments are performed.

This criticism applies to the study of adjuvant lipotropic agents described in this communication. It would not be surprising if these substances were of value after hepatic homotransplantation, since they are said to prevent (or facilitate recovery from) liver injury due to other causes,[5,19] an effect which Schwarz, Stesney, and Foltz[21] attribute in part to tracer quantities of selenium in the sulfur-containing amino acids. The additional factor of irradiation was introduced in those animals receiving S^{35}-methionine, although the calculated dose to the liver or whole body was far too small to be of predictable biologic importance. With testing, the lipotropic materials had no measureable influence on azathioprine hepatotoxicity, did not potentiate survival after homotransplantation to the otherwise untreated host, and did not cause a statistically significant prolongation of posttransplant survival when combined with azathioprine. Nevertheless, it is interesting that the longest average survival after homotransplantation to azathioprine treated recipients was in those 4 groups which also received L-methionine or S^{35}-methionine, a finding which was uninterpretable because the range of results was so great in every group. This may have been due to the system of testing, in which the variables of uncontrolled donor-recipient selection were of far greater importance in the individual experiment than the therapeutic variable being examined.

Although the early recovery after liver homotransplantation has many hazards, chronic convalescence is no more difficult than after the transplantation of less complicated organs. Indeed, the frequency and rapidity with which dogs could be withdrawn from immunosuppression without an ensuing fatal rejection is remarkable. Liver homografts in 5 of 6 dogs which had therapy stopped after 116 to 123 days have provided life-sustaining function for 63 to 204 subsequent days and the cause of death of the sixth animal was from other causes than progressive hepatic failure. The consistency of this state of host-graft nonreactivity and the rapidity with which it seemed to develop exceeds that reported after canine renal homotransplantation.[17,18,23,30] The explanation for this is not apparent, but conceivably the large antigenic mass could play a role or, alternatively, perhaps the liver with its enormous regenerative capacity is simply capable of sustained function in the face of continuing but minimal chronic rejection. Findings in the serial biopsies obtained after discontinuance of therapy do not support the latter hypothesis, but the amount of histologic material available is still too scanty to warrant conclusions. It is also important to note that cessation of therapy was not followed by a graft-host reaction. None of the animals developed "secondary homologous disease" and the shortened red cell half-life noted in the early postoperative period did not recur.

The correlation between liver chemistry determinations and the pathologic findings in the homografts was excellent. In the untreated animal, the abnormalities of function and the anatomic injury were both progressive until death. Administration of azathioprine delayed the onset of rejection in virtually all animals but in most dogs dying within 7 to 15 days the histologic and biochemical alterations were indistinguishable from those of the control experiments. From this time onward, most of the livers appeared to have survived the first assault and were settling down to repair and regeneration; delayed death was commonly due to pneumonitis or other nonhepatic causes. Phagocytic activity of Kupffer cells and macrophages was prominent for several months. The centrilobular localization of the original necrosis seemed responsible for the most conspicuous features of healing, including the centrizonal features of healing, including the centrizonal bile canalicular dilatation and cholestasis, the connective tissue bridges between the central veins, and the regenerating nodules which sometimes contributed to a pseudolobular pattern.

Histologic evidence indicting a vascular component of hepatic rejection was particularly sought in view of previous hypothesis that a reduction in blood flow was immunologically induced at either the sinusoidal level,[13,25] or more proximally in the larger arteries of the portal tracts.[14] The findings in the present study are compatible only with the former possiblity. There was evidence in some of the electron micrographs of damage to the sinusoidal endothelium following contact with host lymphoid cells, a finding analogous to that reported in renal homografts,[11,20] and one which could plausibly explain the centrilobular localization of the hepatocyte necrosis. The possibility of occlusion of larger vessels is unsupported by the results reported here, since the degree of tissue injury to the hepatic artery or portal vein was relatively homogeneous, without areas of selection destruction. Furthermore, injury was not a prominent feature in the untreated series and was virtually never seen in the treated animals.

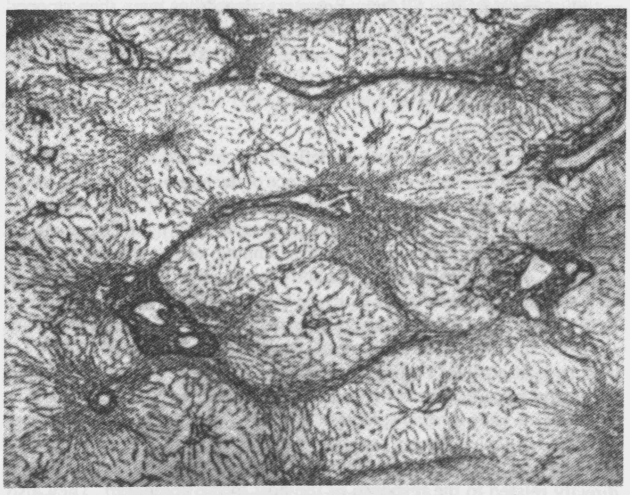

Fig. 11.— Hepatic homograft at 32 days from a dog treated with azathioprine. The reticulin framework of the lobules has collapsed around the central veins and bands of reticulin now connect central veins to each other and to the portal tracts. (Reticulin stain. Original magnification x 20.)

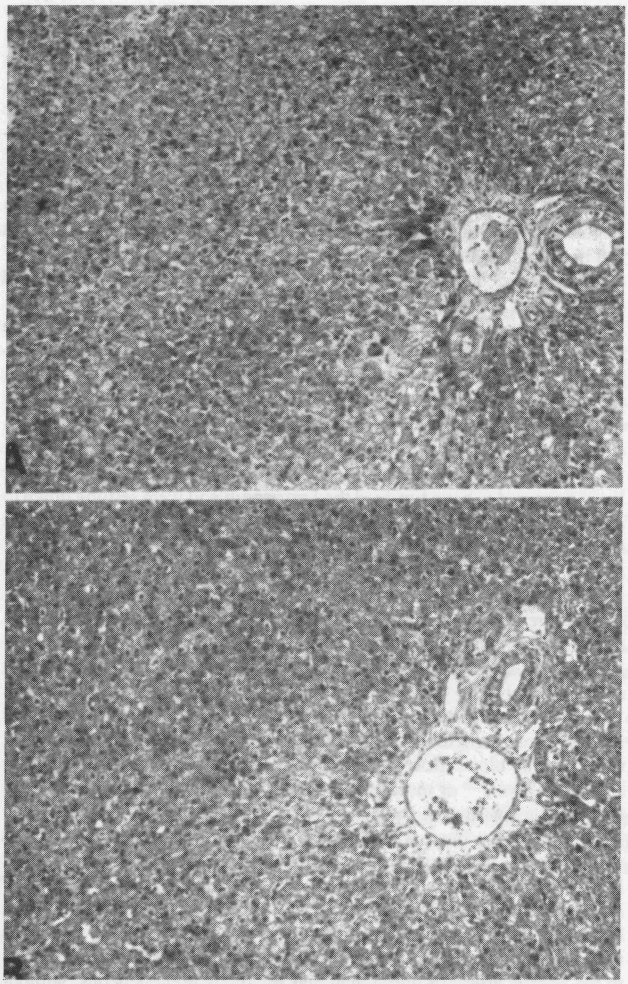

Fig. 12.— Two biopsies from a hepatic homograft. The first (A) was taken after the host had been receiving azathioprine for 120 days. There is evidence of regeneration of hepatocytes at the periphery of the lobules, but no other abnormality. All immunosuppressive drugs were then stopped and 182 days later, 302 days after transplantation, sample B was obtained. The homograft appears normal. (Hematoxylin and eosin. Original magnification x 40.)

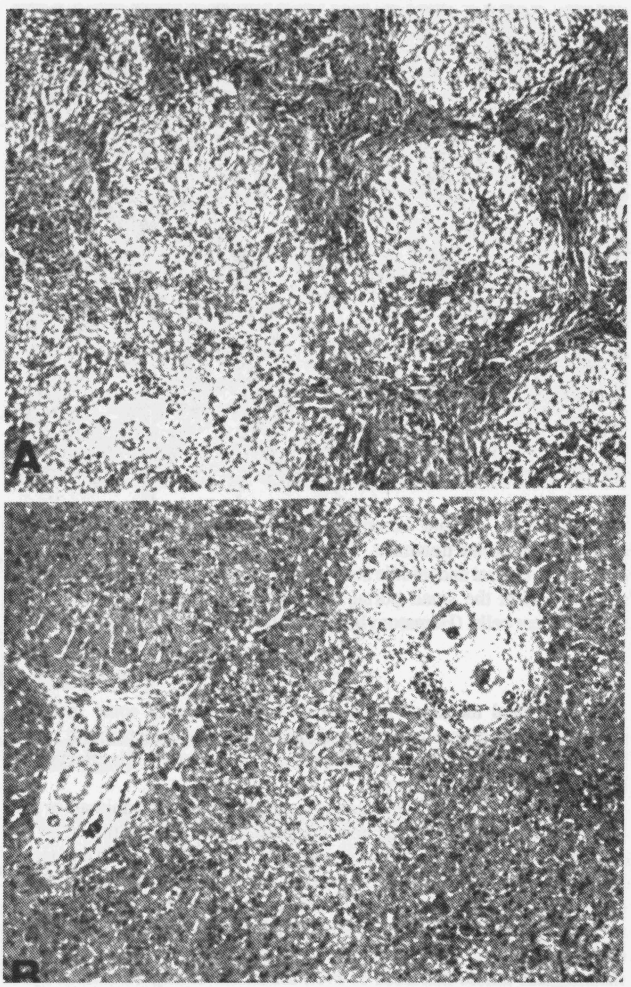

Fig. 13.— Two biopsies from a hepatic homograft. The first (A) was taken after the host had been receiving azathioprine for 121 days. The lobular architecture is distorted by thick bands of connective tissue which link portal tracts to each other and to central veins. Hepatocytes in the pseudolobules of regenerating liver contain much lipid. Azathioprine therapy was then discontinued, and 77 days later, 198 days after transplantation, the second biopsy (B) was taken. There has been a striking improvement in the general liver architecture. Connective tissue bands are no longer so obvious and the liver cells look more healthy. (Hematoxylin and eosin. Original magnification x 40.)

An attempt was also made to define those histologic abnormalities in the host which were related to the presence of the homograft, since varying interpretations have been published. From our laboratories[1,25] findings described after homotransplantation to unmodified recipients included lymph node enlargement with cortical thinning, decreased follicles, and increased numbers of lymphocytes and plasma cells. These alterations, as well as histologic abnormalities in the bone marrow, kidneys, and lungs, were though to be part of a "general host reticuloendothelial response to the antigenic stimulus of the homograft." McBride and associates[13] contended that the host changes (with the possible exception of those in the lymph nodes and spleen) were nonspecific, since in their experience most were also present after autotransplantation.

The findings in the present study support McBride's conclusion that changes in organs other than the host's lymphoid tissue are unlikely to be directly related to the response of the host to the homograft. They also, however, provide evidence that a general lymphoid reaction to the hepatic homograft always occurs in the untreated recipient and can be seen in the treated animal when immunosuppression is inadequate. In these circumstances, the spleen and lymph nodes are sites of intense proliferation of large primitive lymphoid cells and later of plasma cells which are contemporaneously found in the homograft. These changes resemble those occurring in the nodes draining a skin homograft[22] and in the spleen and lymph nodes after a renal homograft.[20]

SUMMARY

Without azathioprine therapy, the operative risk with orthotopic liver transplantation is small. Twenty-two of 23 animals survived 2 days or more, and 19 for 6 days or longer. All eventually died of rejection within 10 days. Changes in homograft histology and function were similar to those previously reported, with cellular infiltration and hepatocyte necrosis which was heavily concentrated in the centrilobular areas. In individual experiments, there was little evidence of immunologically induced segmental hepatic arterial or portal venous occlusion; hepatocyte loss was homogeneous, and fibrinoid vascular lesions were uncommon. There was, however, some evidence of damage to the sinusoidal endothelium by

adherent mononuclear cells. The changing character of the mononuclear infiltration of the homograft was reflected by widespread proliferation of similar cells in the host lymphoid tissue. Specific changes in other host organs were not noted.

Some of the biochemical and histologic alterations caused by unmodified rejection can also be produced by azathioprine. In 18 nontransplanted dogs, acute rises in SGOT, SGPT, and alkaline phosphatase, unaccompanied by hyperbilirubinemia, were noted within a few days after beginning administration of this agent. Although these abnormalities tended to regress within the 40 day period of observation, more than two thirds of the livers showed histologic evidence of centrilobular hepatocyte damage or necrosis — often with intrahepatic cholestasis, but always without mononuclear cell infiltration. The hepatotoxicity was not prevented by methionine. Weight loss and progressive anemia also occurred. Lymphoid tissue was depleted. The mortality from the toxicity study was 33 percent.

The use of azathioprine to mitigate rejection increased the early mortality after homotransplantation, 32 to 116 dogs dying within the first week (28 percent), most commonly of pulmonary complications. The 84 animals living longer than 7 days had a greatly potentiated homograft survival, exceeding 25 days in 44 dogs, and 50 days in 24. Fifteen animals are still alive from 62 to 324 days postoperatively. Six dogs had all drugs stopped after 116 to 123 days. Only 1 has had a clinically evident late rejection and 5 are still alive from 63 to 204 days later. Three of these animals had repeat biopsies 77 to 182 days after cessation of therapy; one homograft which was normal at 4 months remained so 6 months later, another had an improved histologic appearance, and the third had deteriorated. The longest mean survival was in those animals receiving adjuvant therapy with L-methionine or S^{35}-methionine, but the variability of the results was so great that a statistically significant advantage of these agents could not be demonstrated. Soon after operation red cell survival was decreased, but in chronic survivors there was no evidence of a graft-host reaction.

There was great variability in the vigor of rejection, ranging from the uncontrollable (29 per cent) to the clinically undetectable (23 percent). Most of the animals (49 percent) had some biochemical evidence of rejection which proved to be spontaneously reversible, to a greater or lesser degree, since intensification of immunosuppressive therapy was not required. These findings correlate well with the histologic studies. In virtually all animals, azathioprine delayed the onset of rejection but in those dying in the second and third postoperative weeks, the pathologic stigmas of rejection were very similar to the untreated controls. As in the untreated animals, the number of proliferating large pyroninophilic cells in the host's lymphoid tissues was roughly proportional to the number of mononuclear cells invading the homograft liver. After this time, the predominant histologic features in most animals were those of repair and regeneration, with either absent or relatively minor degrees of continuing destruction. Since the major rejection damage was centrizonal, the healing was most prominent in these areas with interconnecting fibrosis around the central veins, centrilobular bile canalicular dilatation and cholestasis, and pseudolobule formation. In some of the homografts, increased connective tissue was also present in the portal tracts, but in others including the longest survivor there were no residual abnormalities whatever.

In azathioprine-treated animals, damage to the vessels in the homograft portal tracts was found in only one liver. With electron microscopy there was some evidence of damage to the sinusoidal endothelium by adherent mononuclear cells, a finding which could be analogous to that described by Kountz and co-workers[11] in the peritubular capillaries of renal homografts. If immunologically mediated hemodynamic alterations play an important role in liver homograft rejection by interrupting the blood supply to the hepatocytes, it seems most likely that they occur at this intrasinusoidal capillary level rather than in the larger vessels.

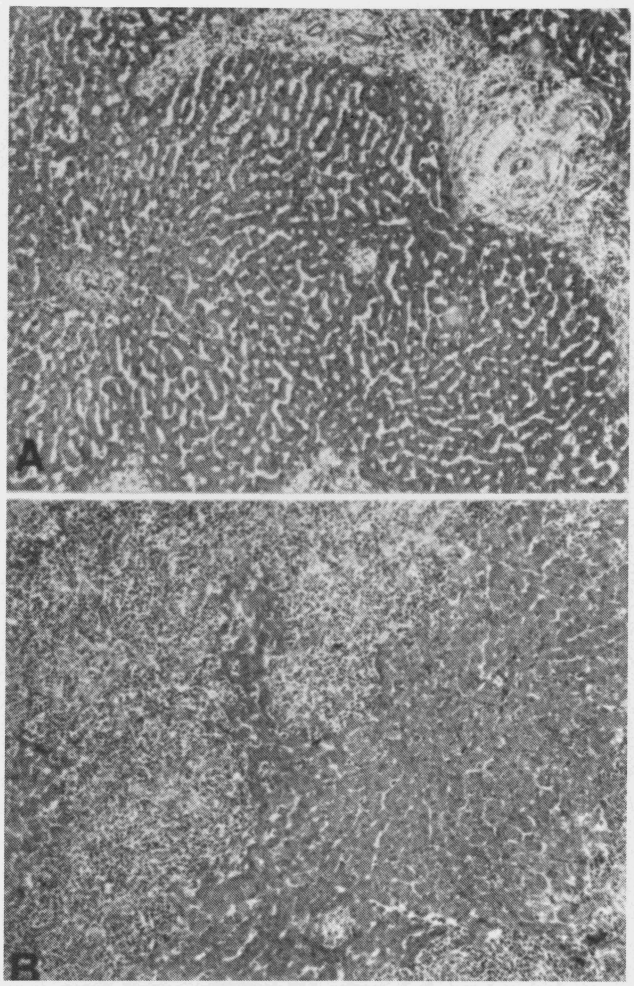

Fig. 14.— Two biopsies from a hepatic homograft. The first (A) was taken after the dog had been treated with azathioprine for 123 days. There is some increase in portal connective tissue, proliferation of small biliary ductules, and a slight cellular infiltration. Treatment with immunosuppressive drugs was stopped, and 84 days later, 207 days after transplantation, the second biopsy (B) was taken. There has been a marked deterioration. The portal tracts are very heavily infiltrated with a mixture of mononuclear and neutrophilic leukocytes. (Hematoxylin and eosin. Original magnification x 40.)

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DISCUSSION

Dr. Francis D. Moore (Boston, Mass.). I apologize for getting up to discuss two papers in a row, but I think this work of Dr. Starzl and his coworkers is the most exciting work in the transplant field since the initial reports by Calne, and later by Zukoski, Murray, and Alexandre, showing the maintenance of intact renal histology with immunosuppression. Now, after a few years and a lot of work, we are beginning to get to the same place with the liver.

Dr. Starzl and his team have furthermore shown a classic thing about transplant research in general. Namely, that they have made an experimental plan, have perfected their own operative procedure, and then have stuck to it, running through a good series of animals with good controls and a careful effort to compare various immunosuppressive regimens.

I rather tend to agree with Dr. Starzl that it is very difficult to make any statistical sense out of minor differences in immunosuppressive regimens because the dog is such a heterogeneous species and relationships are so peculiar. One finds unexpected prolonged acceptance of grafts in many different fields. Nonetheless, Drs. Starzl and Waddell and their group have accomplished more in the way of prolonged survival with liver transplantation than anyone else has, to my knowledge.

As for our own work, we have performed fewer operations and have fewer animals. We agree that early survival can now be attained pretty reliably. Our procedure is slightly different, since we use two end shunts and recently have been using the right renal artery to vascularize the hepatic artery — a nice device, since it avoids the aortic anastomosis and also keeps the pancreaticoduodenal branch intact. Whether this little thing will help us in the long run, I do not know. It is unnecessary in man.

We have seen the very high alkaline phosphatase levels reported by Dr. Starzl in the written manuscript which go "through the roof" with levels of 150 and 250, and may be related to the combination of Imuran and the transplant itself.

We are sufficiently discouraged by the toxicity of Imuran in this particular setting to move over to a different combination, namely, thoracic duct fistula and local x-ray therapy, and I would hope that later in the morning Dr. Murray may show a slide on one of our first thoracic duct fistula kidney transplants, showing its efficacy in lowering the peripheral lymphocyte count.

Finally, one could ask, "Is this problem ready to return to the clinic?" Drs. Starzl and Waddell made a very careful, cautious, and conservatively reported human trial, and we did with one patient. Now that we have learned so much more, are we ready to go back to the clinic yet?

I am not sure of that answer and I will be interested to know what other members of the Society think, because the speed with which we go back to man with liver transplantation is going to have something to do with the public image of surgery in general, as it goes on with the problem of tissue transplantation. Maybe the time has come; maybe it needs another few months or a year, I am not sure.

Again, I would like to congratulate the Denver group on a very outstanding piece of research.

Dr. Richard C. Lillehei (Minneapolis, Minn.). My compliments on Dr. Starzl's paper can only echo Dr. Moore's comments, so I will go on from there.

There is, of course, an alternative to orthotopic homotransplantation of the liver in the animal or man. Some years ago Goodrich and Welch described a method of heterotopic transplantation of the liver in which the donor's liver is left in situ temporarily or permanently. Dr. Karel Absolon of our Department has worked on a similar method in the laboratory for some years, and has written of the many advantages of such a technique. Certainly, it is simpler to place a second liver into the abdomen without removing the recipient's liver. This avoids many of the bleeding and clotting problems associated with hepatectomy, as well as greatly shortening the duration of the operative procedure. These studies of Dr. Absolon culminated in a clinical attempt at heretoropic liver homotransplantation in a 13-month-old boy with proved biliary atresia in the fall of 1964 by Drs. Absolon, Ward O. Griffen, and myself.

The liver for transplantation was obtained from an infant undergoing open-heart surgery for complete transposition of the great vessels. Complete correction by the method of Mustard was not successful and hence it was not possible to remove the cardiopulmonary bypass support despite repeated attempts during a 5 hour period of bypass. Permission was then

asked from the relatives of the infant if the liver could be removed for homotransplantation to the child with biliary atresia. This request was freely granted and the recipient was brought to the operating room and prepared for surgery while the donor was maintained on bypass and the liver removed. At the time of removal of the liver, bypass had continued beyond 8 hours.

The liver was placed in the left lower quadrant of the recipient and circulation re-established by anastomosis of the hepatic artery and inferior vena cava of the transplant to the common iliac vessels of the recipient. The inferior vena cava above the transplant was tied off, while the portal vein of the transplant was anastomosed into its own inferior vena cava to allow decompression of the transplanted liver. The gallbladder of the transplant was anastomosed end-to-side to a loop of the recipient's jejunum. The common bile duct of the transplant was tied off distal to the cystic duct. The recipient's liver was not disturbed. Following this procedure, the recipient's jaundice virtually cleared. The child died on the thirteenth postoperative day from septicemia resulting from a bile fistula due to slough of the common bile duct of the transplant where it was tied off. At autopsy, the transplanted liver appeared histologically normal. Thus we believe that such a technique deserves consideration in future liver transplantation in the human.

Finally, the suitability of other organs for transplant taken from patients undergoing open-heart surgery who cannot be weaned from the support of cardiopulmonary bypass should be emphasized. We, Drs. Aust, Kelly, and myself, have on several occasions used kidneys from such patients for successful homotransplantation after consent from the cardiac surgeon and the next of kin of the patient. In every instance the homotransplanted kidneys have functioned immediately despite periods of cardiopulmonary bypass lasting up to 11 hours before transplantation was performed.

Dr. Starzl (closing). I would like to thank Dr. Moore for his very gracious comments. I suppose the decision about the clinical application of this method will have to rest with the circumstances within any institution contemplating this and the consciences of the people involved.

I would also like to thank Dr. Lillehei for his comments, and because I know of the case I would like to point out one very interesting feature of the Minneapolis transplant. That is, that the transfer was from a donor of A blood type to an O recipient, a violation of the usual rules of tissue transfer, but this did not prevent good function of their homograft.

Antilymphocyte serum (ALS) or globulin (ALG) permitted the prolonged survival of canine renal and hepatic grafts. The results were better with livers than with kidneys. At the time of reporting, four canine hepatic recipients had lived for more than 50 days postoperatively, and although no further treatment was given after 20 postoperative days, two of these animals survived for six and 13 months. The report also described the first human trials of heterologous ALG. All five of the authors subsequently became division chiefs, departmental chairmen or both.

The use of heterologous antilymphoid agents in canine renal and liver homotransplantation and in human renal homotransplantation

Surgery, Gynecology & Obstetrics, 124: 301-18, 1967

Thomas E. Starzl, Thomas L. Marchioro, K. A. Porter, Yoji Iwasaki, G. James Cerilli

The potential value of heterologous antilymphoid serum as an immunosuppressive agent has been known for a number of years. However, the first reference to testing of antiserums for homograft protection was by Woodruff, who later published with Anderson (20, 21) detailed observations of the effect of these agents used alone or in combination with thoracic duct drainage. Subsequent valuable studies have been contributed by Waksman, Gray (2), Monaco (9, 10) and their respective associates and by Jeejeebhoy, Nagaya and Sieker, and Levey and Medawar (6, 7). The experimental model for the foregoing experiments consisted of skin grafting in mice, rats, or guinea pigs which were treated with antiserums raised in rabbits. Except for our own brief report (14) and those of Abaza, and Mitchell and their colleagues there are no published accounts on the use of antilymphoid serum for the protection of whole organ homografts.

The present study is a further evaluation of the importance of this kind of immunosuppressive therapy for the protection of whole organ homografts in dogs, as well as an account of its subsequent application in humans. Antiserums against the lymphoid tissue of both species were raised in horses, characterized for toxicity and antibody content, and rendered to globulin as fully described elsewhere (4). The test organs in the canine experiments were the kidney and liver. The clinical patients had renal homotransplantation.

METHODS

Canine experiments. One hundred and thirty-four mongrel dogs weighing 10 to 25 kilograms were immunized against hepatitis and distemper and used as homograft recipients. Operations were performed under sodium pentobarbital anesthesia combined with the tranquilizer phencyclidine hydrochloride. Renal transplants were accomplished by transferring the donor kidney to the contralateral recipient iliac fossa, with anastomoses of the renal artery to the proximal end of the cut common iliac artery, the renal vein to the side of the common iliac vein, and the ureter to the bladder. Bilateral recipient nephrectomy was always carried out. Orthotopic hepatic transplants were performed as previously described (15). Before and after both kinds of homotransplantation, blood urea nitrogen, serum bilirubin, alkaline phosphatase, serum glutamic oxalacetic transaminase, serum glutamic pyruvic transaminase, and complete hematologic studies were obtained at frequent intervals. The development of canine antibodies against horse serum was monitored by determination of precipitin titers (4).

Animals which died after transplantation were autopsied and the tissues examined by light microscopy. In a number of experiments, biopsies were taken at varying intervals after transplantation and examined both with light and electron microscopy.

Antilymphoid plasma. — The 3 kinds of antilymphoid agents were obtained from immunized horses as described previously (4). The first was unabsorbed antilymphoid plasma with a leukoagglutinin titer of 1:16 to 1:256. The dose of 1 to 4 milliliters per kilogram was given intraperitoneally every 1 to 3 days for 5 to 30 days before renal homotransplantation and by similar schedule during the first 20 or 30 postoperative days. After this time, the intervals between injections were increased to 4 to 30 days or therapy was stopped. The plasma was toxic. Of 36 dogs entered into the experiment, 11 died before operation. Twelve of the remaining 25 definitive test animals had thymectomy 7 to 20 days before institution of plasma therapy.

Antilymphoid serum.— The second product tested was antilymphoid serum with a titer of 1:32 to 1:128. This was obtained from 4 of the same horses, but it was prepared from coagulated blood and partially absorbed against 10 per cent dog red cell pack. Six dogs received renal homotransplantation with a comparable intraperitoneal dose schedule as that described for plasma. In addition, 6 other animals received kidneys with intraperitoneal antiserum therapy starting on the day of transplantation.

The same antilymphoid serum was given intraperitoneally to 9 recipients of orthotopic liver homografts, beginning 1 to 26 days before operation. One of the pretreated dogs received no further therapy after transplantation, but the others had additional postoperative injections.

Antilymphoid globulin.— The third kind of antilymphoid agent

tested was crude globulin which was precipitated from immune horse serum with ammonium sulfate after absorption of the serum with dog red cells, and in some instances with dog serum, kidney, and liver. Many of the batches used were concentrated by lyophilization. The leukoagglutinating titer of the reconstituted injectate was 1:512 to 1:1,024. The subcutaneous dose schedule was 0.2 to 0.5 milliliter per kilogram per day. The effectiveness of immunosuppression was evaluated both with renal and orthotopic liver homotransplantation.

For 25 kidney transplantations pretreatment of 7 to 13 days was provided for 12, and therapy was begun on the day of operation in the other 13. In 4 of 9 dogs receiving liver grafts therapy was started on the day of transplantation, and the other 5 had 5 to 26 days of pretreatment. In the recipients of both kidney and liver homografts, therapy was stopped from 35 to 60 days after transplantation.

Another 4 dogs received renal homotransplantation after pretreatment for 60 days. No postoperative therapy was given to these animals.

Antilymphoid globulin and azathioprine. — Crude globulin was given to 14 dogs, beginning on the day of operation. Postoperatively, 1 milligram per kilogram per day azathioprine was added.

Control studies. — The survival of non-treated control animals previously reported from our laboratory was 11.3 ± 4.6 standard deviation (SD) days after renal homotransplantation (14) and 7.1 ± 2.2 (SD) days after orthotopic liver transplantation (15). New control animals were prepared for the present study with 7 renal and 10 orthotopic liver transplantations. The results were not different from those in the older series for which reason both old and new groups were combined for statistical comparison with the results using antilymphoid therapy.

Four other control animals were treated for 30 days with crude globulin prepared from the serum of nonimmunized horses. The dose of protein was the same, approximately 30 milligrams per kilogram per day, as with the globulin from immunized horses. Renal homotransplantation was then performed, and the subcutaneous globulin administration continued daily until death.

Renal homotransplantation was performed in 7 dogs treated only with 1 milligram per kilogram per day azathioprine.

Data comparison.— The various operations were performed over a period of almost 1-1/2 years with variable periods of available follow-up. In order to avoid bias in the event of unusually long survival and to allow comparison of series studied at different times, credit for survival of any animal was limited to 70 days.

The variously tested antilymphoid substances differed in their titer, degree of purification, and extent of absorption with tissues or red cells from the lymphoid donor species. Therefore, an effort was made to relate the results by means of a unit system which was based upon leukoagglutinating titers of these different agents. If 1 milliliter of either horse plasma, horse serum, or crude horse globulin had a titer of 1:128, it was said to contain 128 units. With a titer of 1:1,024, 1 milliliter was considered to contain 1,024 units. It is recognized that such a unit nomenclature is arbitrary and that the therapeutic efficacy may not be directly related to the titer as the system implies.

Clinical studies. Twelve patients have been given intramuscular antihuman immune globulin which was prepared from the serum of horses immunized with the lymph nodes, thymuses, and spleens of fresh cadavers (4). After absorption of the horse serum with human red cells and serum, precipitation with ammonium sulfate, and lyophilization, the reconstituted product had a leukoagglutinin titer of from 1:8,192 to 1:16,384. The single doses of 4 milliliters provided 450 to 2,100 units per kilogram. The protein content was 7 to 9 grams per cent. The development of human antibodies against horse protein was monitored serially with precipitin tests. The titers of hemagglutinins against sheep red blood cells were also followed, and

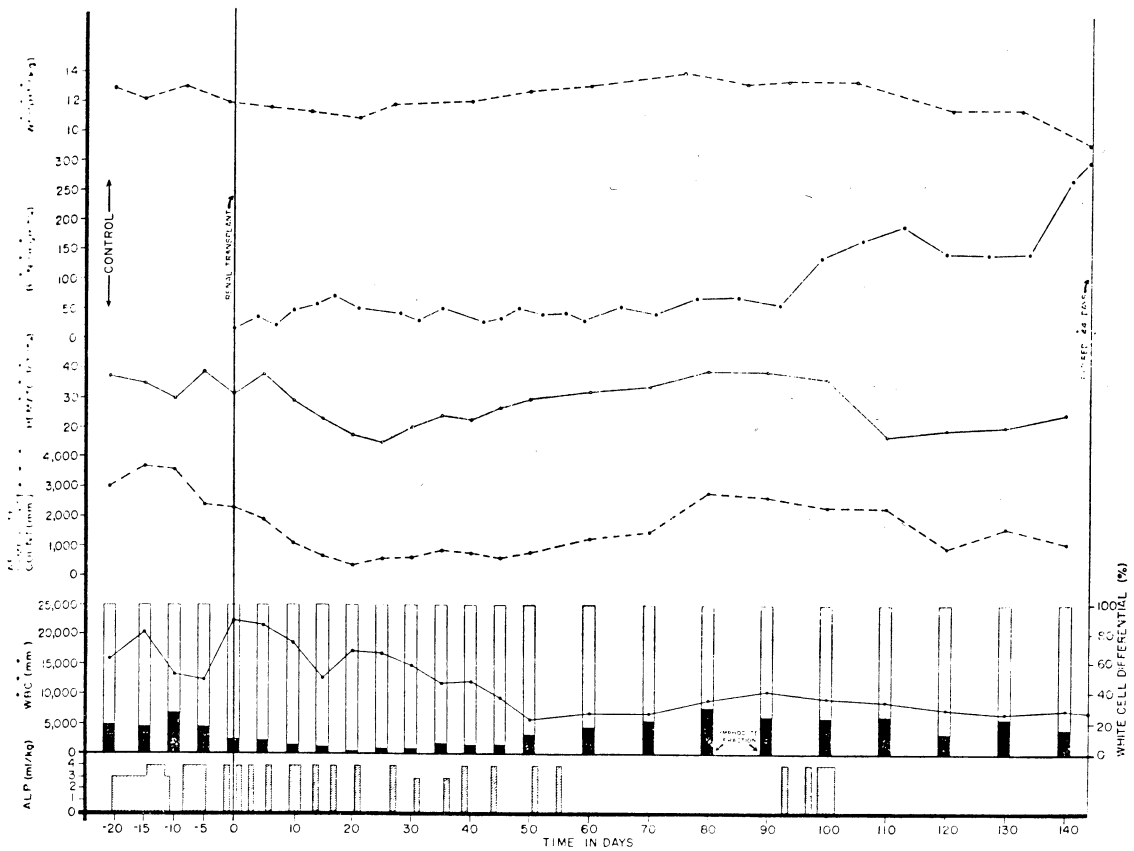

Fig. 1.— Course of a dog after renal homotransplantation, employing antilymphoid plasma (ALP) for immunosuppression. Note the weight loss and anemia caused by both an early and late course of therapy. Rejection was slow to occur after cessation of treatment. Note the minimal change in the lymphocyte count when the plasma was given during the preoperative period.

intradermal skin tests with 0.1 milliliters injectate were performed every 2 weeks. The response to antilymphoid globulin of nontransplanted uremic individuals is described elsewhere (4).

Eight patients with terminal renal failure were treated with immune globulin. In 7, the horse globulin was given for 5 or 6 days in advance of renal homotransplantation as well as postoperatively. In the eighth patient, pretreatment was for 35 days. After operation, injections were continued daily for 1 to 2 weeks, then every other day for 2 to 3 weeks, and finally reduced to twice a week until the fourth month after which 1 injection per week was given. Two of these patients had transthoracic thymectomy prior to transplantation. All 8 kidneys were obtained from blood relatives. There were 2 mothers and 6 siblings. The donor and recipient ages ranged from 21 to 45 and from 16 to 36 years, respectively.

In contrast to the animal experiments, horse globulin was never used as the sole immunosuppressive agent. Azathioprine was started on the day of operation and continued thereafter in daily doses of 5.2 to 0.5 milligram per kilogram per day. During the first month, prednisone was added if needed for control of rejection. After this time, when the antilymphoid globulin therapy was being reduced, prednisone was started in doses of 0.3 to 0.67 milligram per kilogram per day for patients who had not had rejection. Actinomycin C in intravenous doses of 400 micrograms and local homograft irradiation were used irregularly if and when rejection occurred.

To evaluate the effect of the globulin therapy, comparisons were made with comparable patients treated in the past at the University of Colorado in whom survival of at least 63 days had been attained. The control patients of intrafamilial homotransplantations were divided into 3 groups of 25, 13, and 22 each. The first series of recipients, treated in 1962 and 1963, had received azathioprine only until the onset of rejection at which time prednisone was added. The second group received high dose steroid therapy from the day of operation onward in addition to azathioprine. The third group differed in that smaller quantities of prednisone, usually 0.5 milligram per kilogram per day, were started on the day of operation. This steroid dose was increased only when required to treat rejection. In all 3 groups, attempts were later made to reduce the prednisone dosage as rapidly as possible. Since inclusion in the control groups was contingent upon survival for 63 days, unfavorable patients were automatically eliminated. Thus, 7, 1, and 3 patients who were unsuccessfully treated with the respective protocols for less than this period were excluded. An additonal patient in group 2 died on the last day but is included.

Lymphocyte antigens were analyzed by Terasaki and his associates in the donors and recipients from the 3 control groups as well as the antilymphoid series. This information became available retrospectively in 21 of the 25 patients of group 1 and 10 of the 13 patients in group 2. It was obtained prospectively for all patients of group 3 and of the antilymphoid series. In the last series, there were good, average, and poor matches in 3, 3, and 2 patients respectively. Statistical comparison performed by Terasaki revealed no significant differences in the quality of antigen compatibility among the donor-recipient combinations in 4 groups.

For each of the 3 control groups and for the antilymphoid series, the events in the first 63 postoperative days were compared by determining the average daily blood urea nitrogen, creatinine, creatinine clearance, white blood count, lymphocyte differential, lymphocyte count, azathioprine dose, and prednisone dose. From this data it could be determined if the function in the antilymphoid patients was as good as in the previous series, if this function had been achieved with greater or smaller doses of standard immunosuppressive agents, and if there were consistent differences in the peripheral white cell differential and total counts in the various groups.

Late homograft rejection.— Three patients had received maternal, cadaveric, and maternal kidneys respectively, 11, 7, and 5 months previously. In each, there was deterioration of renal function when the prednisone dose was reduced below 1.3, 1.7, and 1 milligram per kilogram per day, in the first, second, and third patients. In each instance, 4 milliliters of antilymphoid globulin with a titer of 1:4,096 to 1:16,384 were instituted every other day or twice a week, and the prednisone dose was then attenuated.

Secondary cadaveric homotransplantation.— A 23 year old girl with removal of a failed homograft 31 months after transplantation from a nonrelated living donor received 2 cadaveric kidneys 5 and 8 weeks later while being treated with antilymphoid globulin, azathioprine, and prednisone. The first cadaveric homograft which began to excrete urine after a week was removed as an emergency 2 days later because of acute renal vein thrombosis. She had a cardiac arrest during bronchoscopy and died 8 days after placement of the second cadaveric kidney which did not function. Since evaluation of therapy was impossible under these circumstances, this patient will not be discussed further.

RESULTS

Canine experiments. Antilymphoid plasma.— The 25 dogs treated before and after renal homotransplantation with doses of 64 to 512 units immune plasma had a survival of 28.2 ± 22 (SD) days, a significant ($p < 0.01$) improvement over that of controls. The mean figures are somewhat

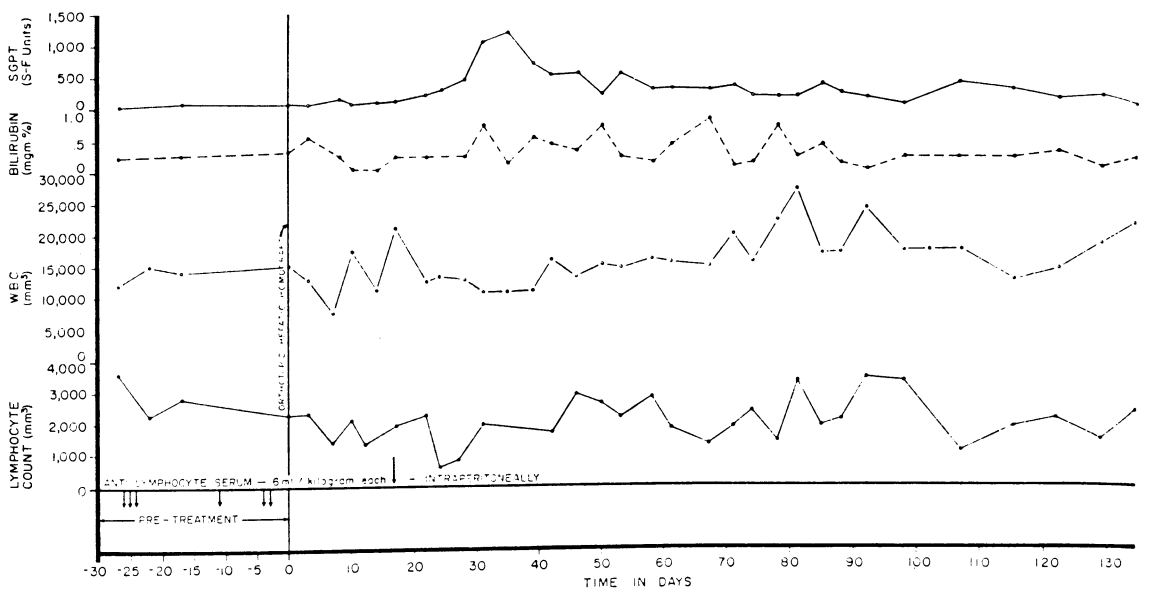

Fig. 2.— A dog which received an orthotopic liver homograft after 6 intraperitoneal injections of antilymphoid serum (ALS). No postoperative therapy was ever given. Note that the lymphocyte count was little changed. The dog is still alive.

lower than those previously reported (14) because survival credit for any individual animal was limited to 70 days. The 12 dogs with thymectomy lived for 26.4 ± 22 (SD) days compared to 29.8 ± 24 (SD) days for the other 13, a difference which was not significant. Of the 25 animals, 18, 11, 9, and 6 survived for at least 15, 20, 30, and 50 days respectively. The 2 longest survivors lived for 95 and 144 days.

During the early postoperative period when injections were given every 1 to 3 days, the animals lost weight. They usually became profoundly anemic, a complication apparently attributable at least in part to the high hemagglutinin titers in the immune plasma (4). Later when therapy was either reduced or stopped, renal failure developed, usually rather slowly, in the longer surviving animals (Fig. 1).

Antilymphoid serum. — Weight loss and anemia were also observed with intraperitoneal antilymphoid serum administrations, but there were no preoperative deaths. The 6 dogs with renal homotransplantation which received both preoperative and postoperative serum in doses of 64 to 512 units survived 27.2 ± 13 (SD) days, a statistically significant ($p < 0.01$) immunosuppressive effect. Five, 4, and 2 animals lived at least 15, 20, and 30 days respectively. The longest survival was 49 days.

In contrast, although 1 of the 6 animals treated beginning at the time of renal homotransplantation lived 38 days, the others all died in less than 15 days. Mean survival was 12.8 ± 12.5 (SD) days, a value which was not significantly better than nontreated controls.

After orthotopic liver transplantation 5, 4, 3, and 2 of the 9 animals lived for more than 15, 20, 30, and 50 days respectively. The mean survival, with a limitation of maximum survival credit to 70 days for individual dogs, was 26.8 ± 26 (SD) days, indicating a statistically significant ($p < 0.05$) prolongation of life. Moreover, 2 of the animals are still alive and in good health after 5 and 6 months.

Two important observations were made in these 2 long-surviving dogs. One animal received only 6 injections of antilymphocyte serum over a 26 day preoperative period. After liver transplantation, no further therapy was given. A definite lymphopenic effect was not produced (Fig. 2). The second animal, which had pretreatment for 1 month, received postoperative serum treatment for only 20 days. After stopping therapy, there was a striking lymphocytosis (Fig. 3), despite which overt rejection has not occurred.

Antilymphoid globulin.— The dogs received 100 to 500 units of globulin subcutaneously. Gradual weight loss was not uncommon. A few batches of globulin caused anemia which developed slowly over a period of several weeks, but this was an unusual complication.

The 12 dogs treated both before and for 2 months after renal homotransplantation had a postoperative survival of 22.9 ± 23 (SD) days, a statistically significant ($p < 0.02$) prolongation of survival. Five, 4, 3, and 3 animals lived for more than 15, 20, 30, and 50 days respectively. In contrast, the 13 dogs treated only after operation had a reduced survival of 14.3 ± 7 (SD) days, a prolongation which was nevertheless statistically significant ($p < 0.05$). This group had 4, 2, 1, and 0 living animals after 15, 20, 30, and 50 days. The slow but progressive late rejection in the animals treated with globulin (Fig. 4) was similar to that described earlier in recipients treated with immune plasma or serum.

The 4 dogs which received kidneys after 60 days of pretreatment but which were not treated after operation all died after 9 to 15 days. Survival was 12 ± 3 (SD) days. There was no statistically significant difference from nontreated controls.

The immunosuppressive effect after orthotopic liver transplantation was more consistent ($p < 0.01$). With limitation of credit to a maximum of 70 days for any dog, the mean survival was 36 ± 30 (SD) days. Five, 5, 4, and 4 dogs lived for more than 15, 20, 30 and 50 days, respectively. The

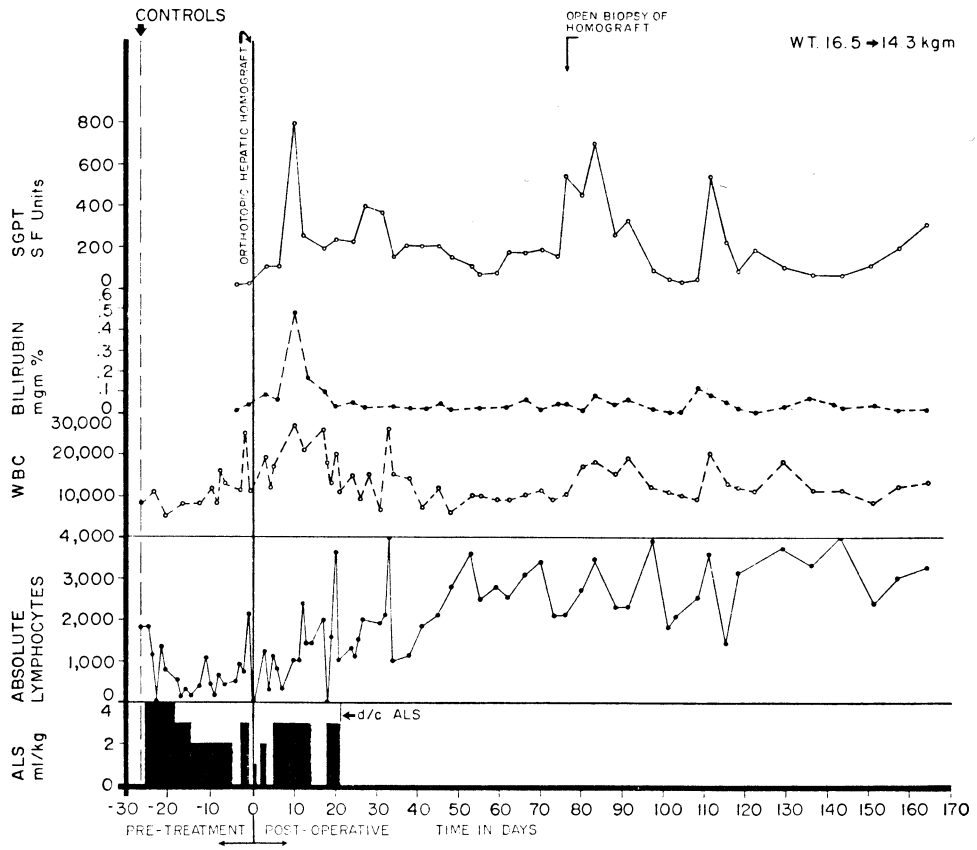

Fig. 3.— A chronically surviving dog whch was treated before and for 20 days after orthotopic liver transplantation with intraperitoneal antilymphoid serum (ALS). Note the pronounced lymphocytosis late in the postoperative period. The animal is in excellent health after almost 6 months.

longest survival, in an animal which is still alive, has been 120 days. The 5 dogs which received therapy both before and after operation did not fare better than the 4 treated only afterward.

As described earlier with immune plasma or serum, the production or maintenance of lymphopenia was not a prerequisite for potentiation of survival after either kidney or liver homotransplantation (Fig. 4). There was not an apparent correlation between the presence or absence of postoperative lymphopenia and the success or failure of therapy.

Antilymphoid globulin and azathioprine. — The 14 dogs which received combined therapy lived for 22.4 ± 19 (SD) days, showing a statistically significant immunosuppressive effect ($p < 0.01$). Eight, 5, 2, and 2 animals survived for more than 15, 20, 30, and 50 days respectively. One of the 2 most chronic dogs died after 60 days and the other is still alive after 100 days. In subsequent statistical comparisons, it should be recalled that globulin therapy was started just before or at the time of operation.

Control studies.— The 7 untreated dogs with renal homotransplantation lived for 9.4 ± 3 (SD) days, with a range of 5 to 14 days. These results, combined with those from 16 comparable experiments performed at an earlier time, gave a mean survival of 10.7 ± 3 (SD) days. After orthotopic liver transplantation in 10 dogs, survival was 6.8 ± 3 (SD) days with a range of 2 to 11 days. The survival of these 10 fresh controls plus 22 more from earlier experiments was 7.0 ± 3 (SD) days.

The 4 animals treated preoperatively and postoperatively with globulin obtained from normal horse serum lived for 14.8 ± 4 (SD) days, with a range of 9 to 18 days. This survival was not significantly better ($p < 0.4$) than that in control animals.

The 7 dogs given 1 milligram per kilogram per day azathioprine survived 20.1 ± 18 (SD) days. Four, 2, 1, and 1 animals lived for at least 15, 20, 30, and 50 days respectively. Thus the doses, although small, had a statistically significant immunosuppressive effect ($p < 0.05$).

Data comparison.— The foregoing data were used to ask several questions of potential clinical importance. To do this, smaller groups were combined into larger series and the significance of differences in results were subjected to the Student t test.

TABLE I.—INCIDENCE OF REJECTION DURING THE FIRST 63 DAYS IN PATIENTS TREATED WITH ANTILYMPHOID GLOBULIN AND IN THE 3 RETROSPECTIVE CONTROL GROUPS

	No.	*Rejection*	*Per cent rejection*
Group 1	25	23	92
Group 2	13	7	54
Group 3	22	18	82
Group 4 (antilymphoid)	8	4	50

The reduced number of rejections in group 2 was evidently due to high dose prophylactic therapy with prednisone.

Is pretreatment valuable? The 18 animals which received either antilymphoid serum or globulin both before and after transplantation had a significantly ($p < 0.05$) better survival than those 19 dogs treated only after operation.

Is combination therapy helpful or harmful? The survival of the 14 dogs treated postoperatively with 1 milligram per kilogram per day azathioprine plus immune globulin was compared with that of the 7 dogs which received the same course of azathioprine alone and with that of 13 dogs which received only postoperative immune globulin. Although the results with the two combined agents were better than those obtained when antilymphoid globulin was used alone in a comparable dose schedule, the difference was not statistically significant ($p < 0.2$). Similarly, the increased survival with the two combined agents in comparison with that using azathioprine alone was not statistically significant.

Is success easier to attain with liver than with kidney transplantation? Survival in the 18 liver experiments involving antilymphoid serum or

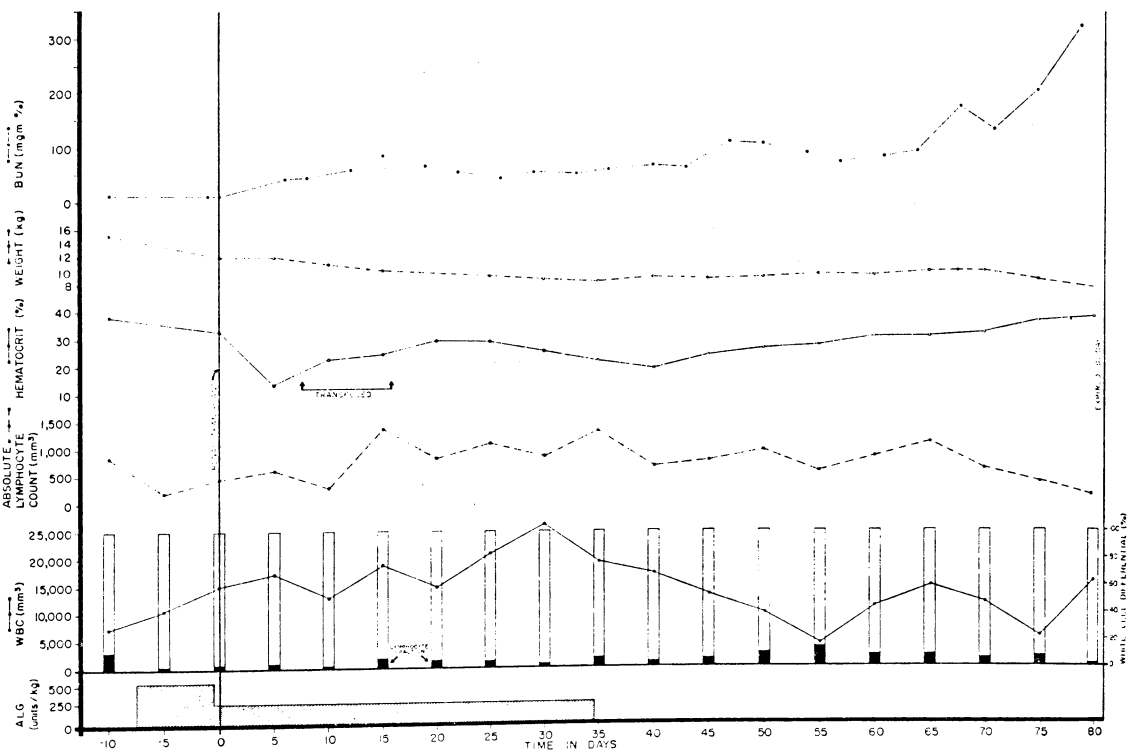

Fig. 4.— Potentiation of canine renal homograft survival with immunosuppression by antidog-lymphoid globulin (ALG). Although the lymphocyte differential fraction was reduced, the total white count was increased and the absolute lymphocyte count was higher postoperatively than during the control period. The early postoperative anemia was apparently due to operative blood loss. Note the slow rejection after cessation of therapy.

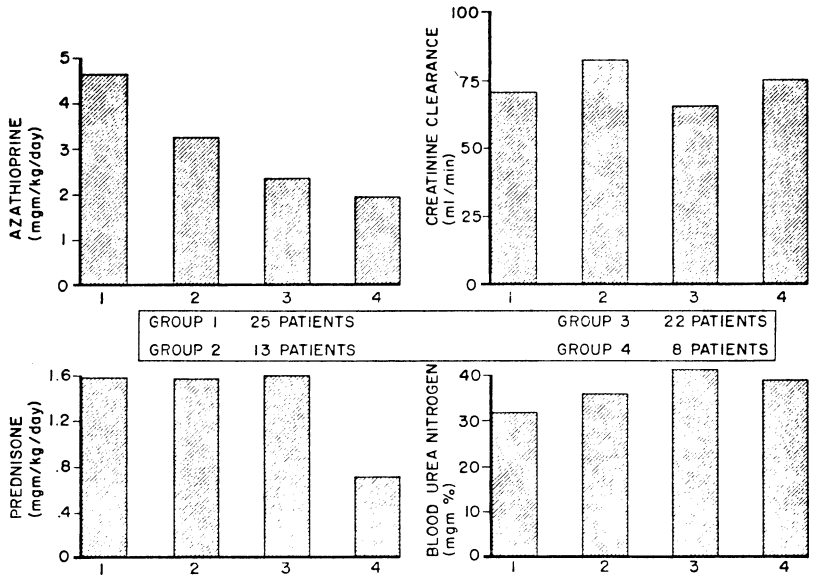

Fig. 5.— Variations in immunosuppression and renal function during the first 63 postoperative days in 4 successive groups of patients who received kidneys from blood relatives. Since the blood urea nitrogen and creatinine clearance were not determined each day, these were compiled on a weekly basis. Those in series 4 received adjuvant therapy with antilymphoid globulin. Note the drastic reduction in average prednisone dose which was achieved in these patients without significant loss of renal function. The progressive diminution of azathioprine dosage in the succeeding series is evident.

globulin was significantly better ($p < 0.05$) than in 37 comparable experiments with renal homotransplantation. The comparison has validity since approximately half of the recipients in each kind of transplantation received therapy both before and after operation, and the other half were only treated following operation.

Pathologic studies.— Ninety-nine of the renal homografts were examined histologically. In dogs treated with antilymphoid substances the features of rejection were not different from those described by Porter and his associates after immunosuppression with other antirejection agents. These included infiltration with mononuclear cells of which 20 to 80 per cent had pyroninophilic cytoplasm. Platelet thrombi and intimal fibrous thickening of interlobular and arcuate arteries were common. Fibrinoid necrosis of arteriolar and arterial walls was present in half the specimens and glomerular capillary basement membrane thickening was found in 27 per cent. Vascular and glomerular changes were rare in either the controls or treated dogs with short survival. The only clear difference in the various test groups was a reduction in the degree of cellular infiltration, the number of cells possessing pyroninophilic cytoplasm, and the incidence of mitoses in those homografts from dogs treated with both low doses of azathioprine and antilymphoid globulin. Two of these 14 kidneys had no evidence of rejection, the only ones completely spared in the entire study.

Similarly, in the 17 hepatic homografts studied either from autopsy or biopsy specimens, either from autopsy or biopsy specimens, the findings were comparable to those previously reported in dogs protected with azathioprine (15). Animals dying before 3 weeks had centrizonal and usually midzonal necrosis, infiltration of mononuclear cells around the portal tracts and central veins, and centrilobular cholestasis. The 6 homografts examined after 3 weeks had variable centrilobular hepatocyte atrophy or reticulin condensation, or even later fibrosis and proliferation of bile ductules in the portal tracts. Five of these 6 livers contained mononuclear cells but in 2 of these the numbers were low.

In all, pyroninophilic cells were sparse. In several of these livers, active rejection appeared to have ceased in contrast to the findings in the homografts of the longest surviving animals after renal homotransplantation when the histologic appearance suggested that none of these kidneys was being tolerated chronically.

The effect of the antilymphoid products on the lymphoid tissue, kidneys, and other recipient organs has been described (4). This included myocardial necrosis and necrotizing coronary arteritis which were also present but in lower incidence in the control animals. Dogs which had renal homotransplantation under antilymphoid therapy alone had hepatic cholestasis or centrizonal necrosis in an incidence of 10 and 4 per cent respectively. When azathioprine was also given, these figures rose to 77 and 61 per cent. This latter finding was probably largely due to the azathioprine which is a known hepatotoxic agent (15), but conceivably the horse protein could have contributed by the synergistic mechanism described by Paronetto and Popper.

Clinical studies. New patients. — All 8 recipients are alive with excellent renal function from 9-1/2 to 14 weeks after transplantation. Their courses during the first 63 days of convalescence are compared to those of the 3 earlier groups in Figures 5 and 8.

From 1962 to 1966 a trend to conservatism with the use of azathioprine was noted. The mean daily dose of 1.92 milligrams per kilogram in the fourth series differed significantly from the means of the first and second ($p < 0.01$) but not of the third group. A significant linear downward trend ($p < 0.01$) was present in all.

In contrast, the average daily dose of steroids in the 3 control groups remained quite stable despite the effort to change the therapeutic protocol. However, the patients in the antilymphoid globulin series received greatly reduced quantities of prednisone. The average daily dose was 0.72 milligram per kilogram, a statistically significant reduction ($p < 0.05$) compared to 1.58 milligrams per kilogram in the pooled control series.

Renal function in all 4 groups was analyzed to determine if the drug reduction described had been at the expense of an increased homograft injury. When the pooled values of blood urea nitrogen, plasma creatinine, and creatinine clearance from the 3 control series were compared with those of the fourth, there was no significant difference in any of the 3 tested variables.

As would be suspected from this data, difficulties of the antilymphoid series with rejection were not serious. This diagnosis was not made during the first 63 days (Fig. 6) in 4 of the 8 patients. In 3 others, a mild rejection crisis was easily reversed. Only 1 recipient had a secondary rise in blood urea nitrogen to over 100 milligrams per cent, requiring protracted high dose steroid therapy (Fig. 7). These results are compared in Table I with those obtained in the 3 retrospective control series. Only in group 2, in which massive steroid therapy was used from the beginning, was there a similar number of patients who escaped early postoperative rejection. The comparison with group 3 is particularly important. The latter patients

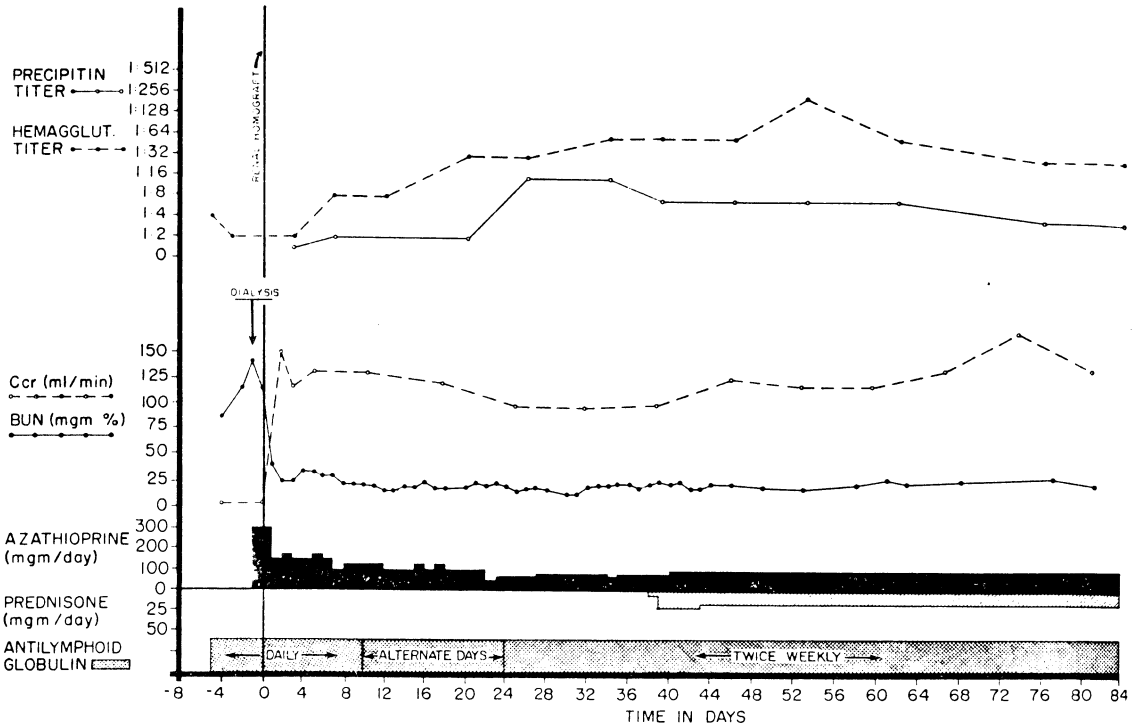

Fig. 6.— Course of a patient treated before and after renal homotransplantation with antilymphoid globulin. No rejection occurred. Note the rises in precipitin and hemagglutinin titers, findings which prompted institution of prednisone therapy. These titers subsequently fell. This patient had a good antigen match with his sibling donor.

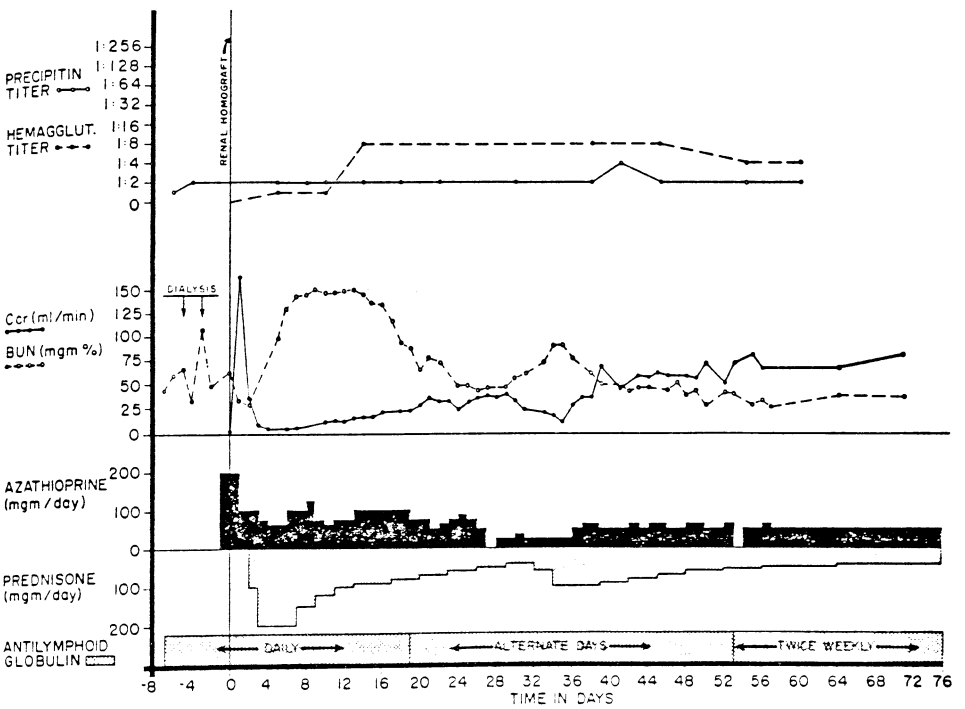

Fig. 7.— The course of the only patient who had a moderately severe rejection among the 8 treated from the beginning with antilymphoid globulin (ALG). The young woman, who had a very poor Terasaki antigen match with her fraternal donor, required large doses of steroids which may have contributed to the relative lack of response in the preciptin and leukoagglutinin titers.

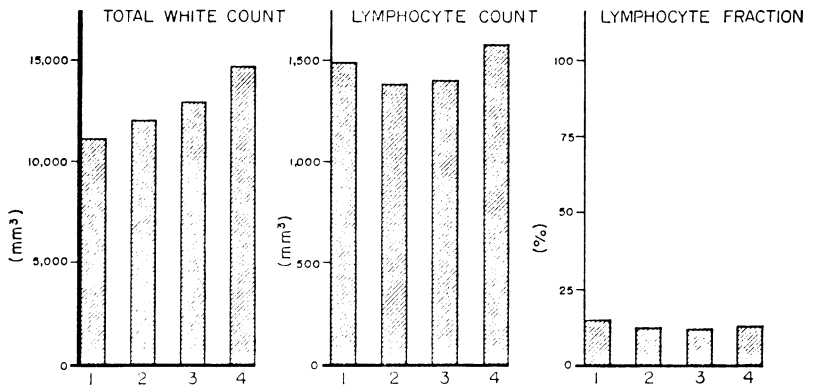

Fig. 8.— Effect of different immunosuppressive regimens upon peripheral white cells. The 4 series are in the same order as in Figure 5. Group 4 received adjuvant therapy with antilymphoid globulin. The higher average total white cell count in the later series was apparently due to greater conservatism with the use of azathioprine. The differences in lymphocyte per cents and total counts were not significant.

all had prospective antigen-typing studies and were treated from the beginning with 30 to 60 milligrams per kilogram per day prednisone, despite which early rejection occurred in 82 per cent, necessitating even higher steroid doses.

Blood changes.— These are shown in Figure 8. With succeeding series there was a distinct linear rise in the average daily total white cell count, probably as the result of the increasingly cautious use of azathioprine described earlier. Due to the variability of the white cell counts, the differences between various pairs of groups were statistically nonsignificant. The per cent of lymphocytes in the peripheral blood was approximately the same in all 4 groups. The average total lymphocyte counts ranged from 1,380 to 1,606 per cubic millimeter, the highest values being from the patients receiving antilymphoid globulin (Fig. 8), but the differences were not significant.

Late homograft rejection.— The clinical courses in these 3 patients during the first 10 to 13 weeks of antilymphoid globulin therapy are summarized in Table II. The 2 patients who had received kidneys from related donors had stabilization of renal function despite very drastic decreases in steroid therapy. In both instances, previous dose adjustments of considerably lesser magnitude had resulted in deterioration of function (Fig. 9). The plasma creatinine and the creatinine clearance were not significantly altered in one patient, and in the other there was slight improvement. There were striking falls in the blood urea nitrogen, which were probably at least partially due to decreased protein catabolism secondary to the steroid withdrawal.

The third patient, who had a failing cadaveric homograft, also had a decline in blood urea nitrogen. Nevertheless, there was a measurable further loss of creatinine clearance with a rise in plasma creatinine during the 13 weeks of steroid withdrawal (Table II).

In all 3 patients, the institution of horse globulin reduced the lymphocyte fraction of the differential count but, because the white count was allowed to increase with adjustments of azathioprine dose, the absolute lymphocyte counts were relatively unchanged.

Evidence of serum toxicity.— Fever as well as tenderness at the injection site was seen in all patients, most severely after the first few injections.

The antihorse-protein precipitin titers rose slightly in all 11 patients. In 10 the maximum titer did not exceed 1:16 and in the other it reached 1:32. Five patients had a precipitin titer which at first rose and then later declined despite continuation of injections (Fig. 7).

Hemagglutinin titers against sheep red blood cells rose in 8 of 10 patients tested, in 6 instances to 1:32 or less, and in the other 2 to 1:256 and 1:512. Secondary declines were documented in 4 patients.

Skin tests, which were obtained in 10 patients, showed an increased reaction in 7. This was usually evident in both the 30 minutes and the 24 hour reading and consisted not only of erythema but also of induration.

Four of the 11 patients had a systemic reaction other than fever. In 1, hives developed. Another became hypertensive for almost an hour. Immediately after injection, in the other 2 patients hypotension developed which lasted for 5 and 30 minutes and which was accompanied by air hunger. All 4 patients recovered without specific therapy and each has subsequently received many more injections without incident.

All patients had quantitative studes of urine protein excretion because of recent demonstration in dogs that antilymphoid globulin can cause renal lesions (4). The 3 patients treated late already had proteinuria before instituting globulin therapy and could not therefore be accurately evaluated. None of the 8 patients treated with globulin from the outset has proteinuria judged by standard laboratory urinalysis. Quantitative analysis of 24 hour specimens revealed protein excretion of less than 400 milligrams per day in each after a follow-up of 2 to 3 months.

DISCUSSION

Two general conclusions with immediate clinical implications seem justified from the information now available concerning antidog-lymphoid products. First, there is an important difference in the response to injections of globulin prepared from the serum of immunized as compared to nonimmunized horses. In the former, development of antibodies to the foreign protein is markedly retarded as measured by the simple and clinically practical precipitin test (4), presumably because of the immunosuppressive qualities of the injectate. The risk of serum sickness is undoubtedly thereby reduced, as the earlier rodent studies of Gray and associates (3) and Levy and Medawar predicted. It is not eliminated, however, since low increases in precipitin titer were often seen in dogs, an observation which may explain the appearance of glomerular lesions after chronic therapy in a significant number of nontransplanted animals as discussed elsewhere (4).

Secondly, although the capacity of the presently available heterologous antilymphoid agents to mitigate rejection of the canine kidney or liver was easily and unequivocally demonstrable in the present series of transplantations, its effect in an individual experiment was unpredictable and weaker than that known to be obtainable with optimal doses of azathioprine. Not only was there a higher incidence of uncontrolled early rejection but also, even after weeks or months, the renal homografts all had evidence of active rejection. It is of interest that more consistent early protection of hepatic homografts was observed, and that in some examined after several months there was little histologic evidence of continuing injury.

The incompleteness of immunosuppression with antilymphoid serum was also evident in the studies of Abaza and his associates with canine renal homotransplantation. Using daily intravenous therapy, they were able to prolong homograft function in 6 of 8 dogs, in 4 of which uremia eventually developed. Maximum survival was 79 days after transplantation and 58 days after removal of the recipient's own kidneys at a second

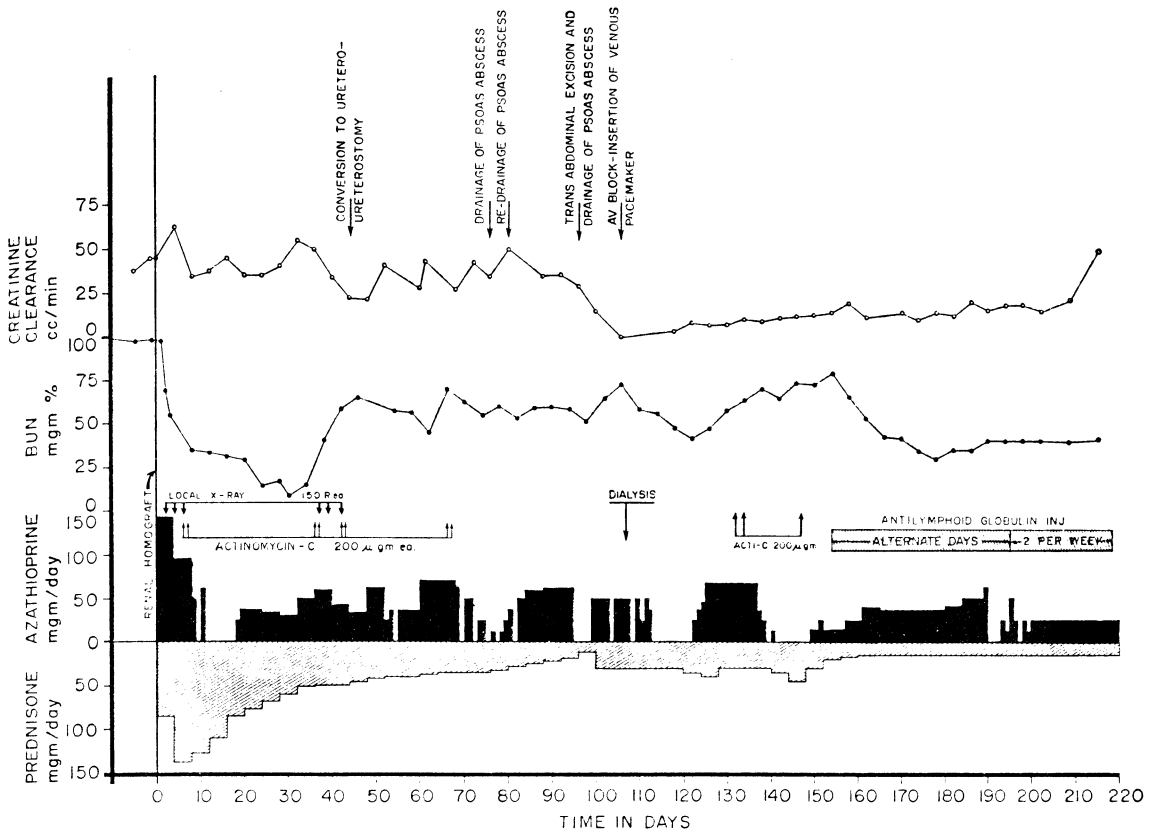

Fig. 9.— Course of patient with a failing renal homograft who was treated late with antilymphoid globulin. During the first 4 postoperative months, there were several potentially lethal postoperative complications which were made more grave by the need for heavy steroid therapy. Efforts to reduce the prednisone dosage resulted in deterioration of renal function. After institution of antilymphoid globulin therapy, renal function slightly improved despite a rapid decrease of the steroid doses.

stage operation. Using sheep antidog-lymphoid serum intravenously, Mitchell and his colleagues reported only 1 of 8 dogs with survival of more than 22 days, but that animal lived for 10 months after simultaneous homotransplantation and bilateral nephrectomy. The consistency of success has thus been less than that reported in inbred rodent experiments by several previous investigators who employed a skin graft test system.

The foregoing information imposed certain restrictions upon the way in which horse antihuman-lymphoid globulin could be clinically evaluated. It was clear that it had to be used as an adjuvant rather than as the primary immunosuppressive drug. For this reason, it became important to know of the physiologic interaction between the antilymphoid substances and the more conventional immunosuppressive agents. This was a particularly important question since an earlier hypothesis of Levey and Medawar (6), subsequently disproved by the same investigators (7), held that the immunosuppressive properties of antilymphoid serum were due to binding of the heterologous antibodies to recipient lymphocytes thereby "blindfolding" and functionally emasculating them. Such a possibility implied that the action of antilymphoid substances was dependent upon a stabile lymphoid population, and that the superimposition of agents which caused a rapid turnover of stem cells might cancel the effect.

The canine experiments with antilymphoid globulin plus azathioprine were therefore critical. It was found that the limited but definite prolongation of survival, which could be achieved with suboptimal therapy using either agent, was slightly increased when the 2 were employed together. Although this improvement was not statistically significant, the really important observation was that survival was not made worse. Moreover, the extent of histologic damage was less in those homografts from dogs provided with combination therapy.

There is evidence that antilymphoid substances can be used to complement the efficacy of other immunosuppressive measures. The studies which are most relevant to the clinical protocol finally decided upon were made by Levey and Medawar (7). They showed a profoundly synergistic effect of adrenal corticosteroids with antilymphoid serum, an observation not dissimilar to that of Woodruff and Anderson (20) who combined antilymphoid serum with thoracic duct drainage in mice. Levey and Medawar (7) have also demonstrated that total body irradiation and immune serum treatment could be used together with benefit, providing the roentgenotherapy was given before exposure to the antigen. When irradiation was used late, well established skin homografts in serum-treated mice were rejected within a few days, long before the expected time.

The influence of thymectomy upon the effectiveness of antilymphoid serum or globulin therapy is more controversial. Monaco and his associates (10) and Jeejeebhoy, using rabbit antiserums in mice and rats respectively, reported far better results when the thymus had been excised. This could not be confirmed in our canine studies, nor in those of Levey and Medawar (7) whose mouse experiments closely resembled those of Monaco and his associates and of Jeejeebhoy. The performance of thymectomy in some of the patients herein reported may not, therefore, have been justified.

Another detail worth additional comment concerns the proper timing for administration of the antilymphoid substances. Careful studies by Levey and Medawar (6) indicated that 1 or 2 intraperitoneal injections were most effective if given 2 days or more after skin grafting and that pretreatment was of considerably less value. Working with a similar mouse system but with therapy for longer periods, Monaco and his colleagues (9) found pretreatment to be distinctively advantageous, a conclusion supported by our canine studies. There is probably no real inconstistency in these results. Monaco's experiments and those herein reported were not

TABLE II.—PATIENTS WITH FAILING RENAL HOMOGRAFTS TREATED WITH ANTILYMPHOID GLOBULIN DURING STEROID WITHDRAWAL

Patient No.	Donor	Months postop. ALG started	Change during 10 to 13 weeks of therapy: B.U.N., mgm. per cent	Creatinine, mgm. per cent	Creatinine clearance, ml./min.	Prednisone, mgm./kgm./day
HD 93	Mother	11	80 → 45	2.4 → 2.4	10 → 12	1.3 → 0.67
HD 102	Mother	5	80 → 40	2.3 → 1.8	16 → 34	1.0 → 0.35
CD 4	Cadaver	7	115 → 75	3.2 → 7.0	5.5 → 3.0	1.7 → 0.23

The changes depicted were over a period of 10 to 13 weeks.
ALG, Antilymphoid globulin.

designed to determine the most important time to give a single or a limited number of injections, but instead to determine if treatment for a number of days before as well as after transplantation was superior to that started just before or at the time of operation. It is important to make this distinction, since pretreatment did appear to be of value in the canine experiments and was accordingly made part of the protocol used for patients.

There is evidence that the clincal regimen developed as a result of these various considerations has been at least of short term value. The 8 recipients in the test series did not have unusually complete antigen matches with their donors and did not appear to enjoy other advantages over previously treated patients. The 8 patients are well from 9-1/2 to 14 weeks postoperatively. In each of the preceding control series there was a 15 to 28 per cent mortality rate during the same period. The quantities of azathioprine and prednisone were appreciably reduced in the globulin-treated patients during the critical first 2 months without the sacrifice of renal function. This was particularly clear, with calculation of the steroid doses, since the total amounts required in each preceding series had been fixed at almost the same level. The significance of these observations is increased by the fact that data from those patients in the earlier series who died before 9 weeks were excluded from the comparative calculations, thereby introducing a bias against the antilymphoid group.

The influence of the globulin therapy upon the courses of the 3 patients with late-failing homografts is less clear. In 2 of these 3 patients, the doses of prednisone were materially reduced without further evidence of kidney injury. It is probable that renal function in such patients can be improved little, if at all, by institution of antilymphoid globulin injections, but it is possible that residual function can be sustained with considerably less prednisone. Benefit of this sort would not be unexpected even in these patients with late-failing grafts since Monaco and associates (9) and Levey and Medawar (7) showed that antilymphoid serum can erase the immunologic memory of presensitized animals.

The means by which heterologous antilymphoid substances mitigate homograft rejection has been discussed elsewhere from the viewpoint of the pathologic changes they cause in the lymphoid tissues of dogs (4) and in light of the information recently provided by Levey and Medawar (7). Although the immunosuppressive effect of these agents has not been fully explained, the evidence is growing that it is contingent upon neither lymphopenia nor lymphoid depletion. The horse serum or globulin used for dogs and patients of the present study caused lymphopenia under appropriate conditions of in vivo testing (4), a fact which is probably of practical importance in deciding whether or not a potent product is being used. Nevertheless, this was often not observed in animals which survived for long periods after either kidney or liver homotransplantation.

In the patients who were treated with 2 or more drugs in addition to globulin, the significance of changes in lymphocyte counts was even more difficult to interpret, particularly when comparing results in the test group with those obtained from earlier series. In a past clinical study which included many patients from the retrospective control groups (16), it was shown that lymphocytosis in the post-transplant period could not be correlated with the presence or absence of rejection. There was, however, a direct correlation between the quantity of steroids employed and the degree of lymphopenia. This important pharmacologic variable was present in both our control and test series. Consequently, more cannnot be said than that the patients treated with antilymphoid globulin had approximately the same number of peripheral lymphocytes as previously treated patients who had received more than twice as much prednisone.

Thus far, discussion has been largely confined to the considerable body of evidence supporting the concept that heterologous antilymphoid globulins may be useful agents in clinical homotransplantation. It is important to emphasize that the extent to which this may be true will be strongly influenced by further studies of clinical toxicity. It was mentioned earlier that glomerular lesions can be produced in normal dogs by chronic adminstration of these substances. Biopsy of the human homografts will be necessary at a relatively early time to determine if similar lesions are present, even though none of the patients now has proteinuria. Such histologic information will guide a decision for or against further therapy in these or future patients.

Furthermore, other features of the patients' responses to horse protein are not yet clear. The modest and often reversible increases in antihorse-precipitin titers have been cited. Moreover, the significance of rises in antisheep red cell agglutinins, which are apparently in response to a Forssman type antigen in the horse protein, is not yet known. Although life-threatening toxicity has not been observed, this possibility has not yet been excluded. It was principally because of this consideration that 4 of the 8 patients in the series of new patients were eventually treated with low dose steroid therapy even though the clinical diagnosis of homograft rejection had not been made.

SUMMARY

Plasma, serum, or globulin were prepared from the blood of horses which had been immunized with canine lymphoid tissues. The respective agents which were rendered progressively less toxic by appropriate absorption and purification procedures were administered by the intraperitoneal or subcutaneous route to dogs which received either renal or orthotopic liver homotransplantation. The survival of animals after either kind of procedure was significantly prolonged compared to that of controls, although in virtually all clinical and pathologic evidence of rejection eventually developed. The best results were obtained if treatment was started several days before operation and continued thereafter. The degree of protection seemed to be greater for the liver homografts than for the kidneys. Combination therapy of anitlymphoid globulin and small doses of azathioprine resulted in slight and statistically nonsignificant increases in survival, but the degree of histologic injury seemed to be reduced.

Although easily demonstrated, the immunosuppressive effect of the antilymphoid products was not strong enough to warrant clinical use as other than an adjuvant agent. Horse antihuman-lymphoid globulin has been used for this purpose in 11 patients in combination with azathioprine and prednisone. Eight of these patients received intramuscular globulin starting before operation and continuing until the present time. Their need for therapy with the standard immunosuppressive drugs has seemed to be reduced without a consequent loss of homograft function. All are well after 9-1/2 to 14 weeks. The other 3 patients had failing renal homografts which had been placed from 5 to 11 months previously. After institution of globulin therapy in the latter patients, steroid doses were reduced. In 2 of the 3 patients, renal function stabilized or improved in the ensuing 10 to 13 weeks, but in the third there has been further deterioration.

The experimental nature of the clinical trial has been stressed in light of the unknown risk of serious toxicity from the injections of horse protein.

This morbidity can be completely assessed only with further observation and by study of homograft biopsies in those patients now under treatment. Wider clinical trial is not recommended until this information has been obtained.

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The kidney was the principal organ used by Calne et al in their first clinical trials of cyclosporine. However, two orthotopic liver transplant recipients also were included. This was one of the most important clinical papers on transplantation ever published. Almost two decades previously, Calne had been one of the most important contributors to the development of baseline therapy with 6-mercaptopurine and azathioprine[9, 10] A description of the use of azathioprine-steroid therapy for liver recipients in England is reproduced in Part III.

Cyclosporin A initially as the only immunosuppressant in 34 recipients of cadaveric organs: 32 kidneys, 2 pancreases, and 2 livers

Lancet, 2: 1033-36, 1979

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Summary

34 patients treated with cyclosporin A received 36 cadaveric organ allografts (32 kidneys, 2 pancreases, and 2 livers). 26 kidneys are still supporting life, 3 after more than a year; the pancreases and livers are also functioning. 20 patients are not receiving steroids, and 15 of these have not had any additional immunosuppressive agents. In these patients infectious complications have not been severe, but a gastroduodenal lymphoma has developed in 1 patient. 6 patients were given 'Cytimun' (a cyclophosphamide derivative) and steroids in addition to cyclosporin A: 5 of these died of infections and 1 also had a lymphoma. 11 patients received additional steroids: 1 of these died from septicæmia and lymphoma. Nephrotoxicity can be avoided by perioperative hydration and forced diuresis. Cyclosporin A is effective on its own and is a very potent immunosuppressive drug. Additional immunosuppressive agents may lead to severe complications.

Introduction

We report here our continuing experience of a pilot study of cyclosporin A (CyA) begun more than a year ago.[1] 32 patients in renal failure, all of whom had received previous blood transfusions, were treated. 31 received first allografts and 1 a second allograft. 1 received a pancreas allograft from the kidney donor. The pancreas was transplanted in an iliac fossa by means of a technique based on that of Dubernard et al.[2] 2 patients were orthotopically transplanted with livers: 1 of these, who had juvenile-onset diabetes, received a heterotopic pancreatic allograft, also by means of Dubernard's technique. All patients and donors were mismatched for A and B HLA antigens; most had two or more mismatches (see table). The DR matching is not yet available.

The starting dose of CyA was 25 mg/kg/day in 7 patients, 10 mg/kg/day in 6, and 17 mg/kg/day in 21. 26 renal allografts are still sustaining life — 3 of them more than a year after transplantation. The pancreases and livers are also functioning. 20 of the patients are off steroids, including 15 of 16 who received no additional immunosuppressive agents at any time. There has been 1 acute rejection crisis with fever and a swollen kidney. Rejection episodes otherwise have presented as impaired renal function. No kidney has been lost through rejection.

Nephrotoxicity

In our first report of this study we were disturbed by the nephrotoxicity of CyA.[1] All the kidneys came from donors with brain death and intact circulations. There have been 6 cases with primary anuria and 9 cases of secondary anuria. 9 of these 15 patients had insufficient histological changes on renal biopsy to explain the anuria. 4 had substantial rejection. We observed primary diuresis with good continuing function in patients who had been deliberately hydrated and given mannitol. This is now our standard policy. 19 patients were treated in this way and had primary diuresis. 3 of these developed secondary anuria with severe rejection on renal biopsy, which in each case responded to a course of steroids. 16 of these patients have renal allografts functioning without steroids and 11 have not received additional immunosuppressive agents.

Infections and Malignancy

Of the 16 patients treated with CyA only, 2 developed self-limiting viral infections — 1 herpes simplex and 1 herpes zoster and herpes simplex. 1 developed cytomegalovirus infection and immunosuppression was changed: CyA was stopped and steroids and 'Cytimum' (a cyclophosphamide derivative) were started. 1 allograft from which repeated biopsy specimens had been taken became infected with bacteria and was removed. 1 patient presented with weight loss and anæmia. A gastroduodenal lymphoma was found at endoscopy and resected. The patient has returned to work free of symptoms. 5 of 6 patients given steroids and cytomun died of sepsis: 1 of these had a jejunal lymphoma which was discovered at necropsy. 1 of 11 patients given additional steroids died of spesis and pulmonary lymphoma. Of the remaining 10, 1 had severe herpes-simplex infection and 1 perforated a sigmoid diverticulum which required a colostomy. Both of these patients recovered.

DETAILS OF TRANSPLANT PATIENTS TREATED WITH CYA

Case no.	Age and sex	Date of transplant	No. of HLA-B mismatches	Starting dose of CyA (mg/kg/day)	Present dose of CyA (mg/kg/day)	Other drugs	Outcome and present function
1	37 M	June 26, 1978	2	25	. .	No	Transplant nephrectomy day 28. Pyelonephritis in graft. On dialysis.
2	30 F	July 1, 1978	3	25	12	No	Discharged day 22. June 28, 1979: serum urea 8·9 mmol/l, serum creatinine 119 mmol/l
3	21 M	July 1, 1978	4	25	12·5	CM	Vascular rejection day 7. Recovered function. Discharged day 53. Sept. 6, 1979: serum urea 13·3 mmol/l, serum creatinine 287 mmol/l
4	27 M	July 14, 1978	3	25	. .	CM Pred	Cytomegalovirus infection and varicella. Died of sepsis at 11 mo. Lymphoma found in jejunum
5	22 F	July 14, 1978	2	25	. .	CM Pred	Marrow aplasia and septicæmia. Died after 139 days
6	46 M	Aug. 4, 1978	2	25	. .	CM Pred	Septicæmia. Died after 55 days
7	52 M	Aug. 4, 1978	2	25	3	CM Pred	No initial function. 1 litre day 24. Function improved with falling dose of CyA. CM stopped at 1 yr. Pred now 5 mg/day. Biopsy at 13 mo: focal scarring and hypertensive changes. At 13 mo serum urea 21·8 mmol/l, serum creatinine 418 mmol/l
8	46 M	Nov. 11, 1978	2	10	5	No	God function. Biopsy at 11 mo: focal scarring and hypertensive changes. At 11 mo serum urea 18·7 mmol/l, serum creatinine 359 mmol/l
9	44 F	Dec. 5, 1978	3	10	5	Pred	Oliguric day 0. Diuresis day 9. Aug. 30, 1979: serum urea 29·9 mmol/l, serum creatinine 490 mmol/l
10	29 M	Jan. 25, 1979	2	10	. .	CM Pred	Diuresis day 1. Oliguric day 4. Good function until death at 8 mo from pneumcystis pneumonia
11	64 F	Jan. 26, 1979	2	10	7	Pred	Diuresis day 1, then oliguria. Good function. Aug. 30, 1979: serum urea 13.8 mmol/l, serum creatinine 135 mmol/l. Pred now 5 mg/day
12	54 M	Feb. 28, 1979	2	17	. .	Pred	Diuresis day 14. Died at 4 mo of septicæmia and penumonitis. Lymphoma found in lungs
13	36 M	March 7, 1979	2	17	17	No	Diuresis day 1. Good function. Sept. 13, 1979: serum urea 10·6 mmol/l, serum creatinine 256 mmol/l
14	57 F	March 19, 1979	1	17	. .	CM Pred	Anuric. No function at any time. Died day 75 from septicæmia and pulmonitis. Candidiasis
15	59 M	April 23, 1979	2	17	12	Pred	Anuric. Diuresis day 10. Good function. Sept. 13, 1979: serum urea 10·9 mmol/l, serum creatinine 136 mmol/l
16	50 M	May 4, 1979	2	17	1·6	No	Diuresis day 16. Good function. Sept. 19, 1979: lymphoma resected from 1st part of duodenum and lesser curve of stomach. Serum urea 6·5 mmol/l, serum creatinine 141 mmol/l
17	31 M	May 4, 1979	1	17	12·5	Pred cover for asthma for 2wk	Diuresis day 1. Good function Sept. 6, 1979: serum urea 19·3 mmol/l, serum creatinine 255 mmol/l
18	39 F	May 19, 1979	1	17	15	No	Diuresis day 1. Good function. Sept. 27, 1979: serum urea 6·9 mmol/l, serum creatinine 122 mmol/l
19	66 M	May 19, 1979	1	17	8·5	No	Diuresis day 1. Good function Sept. 13, 1979: serum urea 18·2 mmol/l, serum creatinine 163 mmol/l
20	12 F	May 30, 1979	2	17	10	No	Diuresis day 1. Good function. Sept 20, 1979: serum urea 6·8 mmol/l, serum creatinine 102 mmol/l
21	37 F	July 10, 1979	2	17	7	No	Diuresis day 1. Good function. Sept. 13, 1979: serum urea 9·9 mmol/l, serum creatinine 181 mmol/l
22	52 M	July 10, 1979	2	17	6	SM 3×1 g	Diuresis day 1. Good function. Sept. 21, 1979: serum urea 23·9 mmol/l, serum creatinine 280 mmol/l

DETAILS OF TRANSPLANT PATIENTS TREATED WITH CYA—(continued)

Case no.	Age and sex	Date of transplant	No. of HLA-B mismatches	Starting dose of CyA (mg/kg/day)	Present dose of CyA (mg/kg/day)	Other drugs	Outcome and present function
23	59 F	Aug. 8, 1979	2	17	5	SM 5×1 g	Diuresis day 1. Biopsy day 42: moderate cellular rejection. Sept. 12, 1979: serum urea 14·7 mmol/l, serum creatinine 182 mmol/l. Improving
24	50 M	Aug. 2, 1979	2	17	5	Pred	Diuresis day 1. Biopsy day 36: moderate cellular rejection. Pred now reduced to 25 mg/day. Serum urea 19·5 mmol/l, serum creatinine 227 mmol/l. Improving
25	17 F	Aug. 5, 1979	2	17	14	Pred	Diuresis day 1. Biopsy day 18: severe cellular rejection. Pred now reduced to 20 mg/day. Serum urea 21 mmol/l, serum creatinine 329 mmol/l. Improving
26	25 M	Aug. 5, 1979	3	17	12·5	Pred	Diuresis day 1. Biopsy day 21: severe cellular rejection. Pred now reduced to 15 mg/day. Serum urea 10·4 mmol/l, serum creatinine 225 mmol/l
27	55 F	Aug. 18, 1979 (+pancreas)	4	17	13	No	Diuresis day 1. Good function. Serum urea 7·5 mmol/l, serum creatinine 97 mmol/l, plasma glucose 5·4 mmol/l
28	29 M	Sept. 4, 1979	2	17	15	SM 4×1 g	Diuresis day 1. Biopsy day 13: moderate cellular rejection. Function now: serum urea 54·4 mmol/l, serum creatinine 664 mmol/l. Improving
29	50 F	Sept. 10, 1979(liver)	Not done	10	8	No	Good function
30	68 M	Sept. 17, 1979	2	17	17	No	Diuresis day 1. Function now: serum urea 18·1 mmol/l, serum creatinine 287 mmol/l. Improving
31	49 M	Sept. 18, 1979	1	17	15	No	Diuresis day 1. Function now: serum urea 26·8 mmol/l, serum creatinine 285 mmol/l. Improving
32	25 M	Oct. 3, 1979(liver +pancreas)	Not done	10	10	No	Good function
33	62 M	Oct. 3, 1979	3	17	17	No	Diuresis day 1
34	44 F	Oct. 3, 1979	3	17	17	No	Diuresis day 1

*All patients except nos. 29 and 32 had renal allografts.
CM=Cytimun, Pred=Prednisolone. SM='Solu-medrone'.

Other Side-effects

All but 2 patients had abnormalities of liver function early after operation. These consisted of raised serum bilirubin and alkaline-phosphatase levels and, in approximately half, raised serum-transaminase levels. The 2 recipients of liver allografts have similar mild derangements of liver function. The abnormalities of liver function tended to resolve as the dose of CyA was reduced. Most patients had an increase in growth of hair on the face and body, and most of the patients with their own teeth developed gum hypertrophy, which tended to get better with time. Tremor was common in the early postoperative phase; usually it was mild and resolved with time; in 3 it was severe.

Renal Function

None of the 26 patients with life-supporting renal allografts has entirely normal kidney function. The lowest serum creatinine is 97 mmol/l and urea 4.1 mmol/l. As a group, those patients still being treated with additional steroids have the worst function, the highest serum creatinine being 470 mmol/l and urea 31 mmol/l.

We have on several occasions noticed an improvement of renal function as the dose of CyA was reduced. Most patients surviving more than six months are receiving 10-12 mg/kg/day of CyA, although 1 is receiving only 1.6 mg/kg/day. 2 grafts showed evidence of rejection when the dose of CyA was reduced.

Discussion

We are still ignorant of the pharmacodynamics of CyA in man. A radioimmunoassay for CyA is being developed, but we do not yet know if fluctuations in absorption and serum concentrations are related to nephrotoxicity and immunosuppressive potency. Most CyA is thought to be excreted in the bile, and since if is fat-soluble, abnormalities of liver function probably interfere with absorption and excretion of the drug. Although an optimum dosage has not been clarified, from our limited experience we feel that 10 mg/kg/day may be too little as an initial dose and 25 mg/kg/day too much. We are getting better results wiht 17 mg/kg/day starting dose. We tend to reduce the dosage slowly but have been reluctant to cut it below 10 mg/kg/day, and CyA has not been stopped in any of the currently surviving patients. This policy is based on our experience with renal allografts in dogs and orthotopic heart transplants in pigs, where rejection occurred in most animals when CyA was stopped, although two pigs treated with CyA for six months are surviving a year after stopping the drug.[3-5]

Powles et al.[6] have used CyA to treat graft-versus-host disease in patients with bone-marrow grafts. They observed rapid recurrence of the cutaneous lesions after withdrawal of CyA therapy. CyA shows striking species differences in its effects on organ allografts. In the rabbit the drug can sometimes be stopped quite early without subsequent rejection,[7] but slow rejection may eventually cause renal failure.[8]

The mode of action of CyA is not understood but it is the first partially selective immunosuppressive drug. It acts at an early stage in the differentiation of T cells[9,10] and possibly on a subpopulation of these.[11] Although the mechanism of nephrotoxicity of CyA is not known, it is reversible and not associated with morphological changes on light or electron microscopy. In view of the good results in patients who were deliberately hydrated and infused with mannitol at the time of operation, we decided after the nineteenth case to hydrate and give mannitol to all patients and to withhold CyA until is was clear that there was a diuresis after 6 h. If the patient's own kidneys were present, a diethylene-triamine-penta-acetic-acid scan was done to determine that urine was coming from the transplanted kidney. If there was no diuresis we were planning to treat the patients with conventional immunosuppression with azathioprine and steroids. This policy of selection has so far not been tested because all 13 patients subsequently managed in this way have had adequate diuresis at 6 h and have then started CyA treatment. It was also decided that if secondary anuria occurred a percutaneous renal biopsy would be done, and if this showed rejection one course of steroids would be given and then the steroids would be stopped. If rejection continued despite steroid treatment, we would either abandon the kidney, stop immunosuppression and remove the allograft, or (if the patient's condition was satisfactory) stop the CyA and change to azathioprine and steroids. In 6 patients allograft function deteriorated and needle biopsies of the transplants showed rejection. All 6 patients responded satisfactorily to additional steroid; and in 3 the steroids have been stopped.

We feel it is most important to avoid adding other immunosuppressive agents to CyA, if possible. A high incidence of infection and two lymphomas wer found in patietns treated with additional drugs. The third lymphoma developed in a patient treated only with CyA. Malignant lymphoma is a well-known complication of conventional immunosuppression.[12,13] 18% of heart-grafted patients with cardiomypathy developed malignant lymphomata.[14] They were treated with high doses of azathioprine, steroids, and antithymocyte globulin. In our trial of 34 cases extending over ony 15 months the occurrence of three lymphomata is disturbing and makes us reluctant to make speculations about the future of CyA. Our experience suggests that in man CyA is the most powerful immunosuppressive agent so far used in the management of patients with cadaveric renal allografts. One of its most attractive features is that is can be steroid-sparing. It is also relatively non-toxic to the bone marrow. Patients do not have difficulty in taking the drug and most are delighted with the lack of steroidal side-effects.

Safe and effective immunosuppression remains an elusive goal. The search for predictable, truly donor-specific immunosuppression has been disappointing. We expect that in the foreseeable future non-specific pharmacological immunosuppression with highly selective agents will be required in clinical organ grafting. CyA is a move in this direction in terms of its selectivity against a subpopulation of lymphocytes, but its side-effects have been more serious than would have been expected from much experimental work in various species. Despite careful assessment in animals, when a drug is first used clinically the patient embarks on an uncertain and possibly hazardous journey. Every effort must be made not to repeat mistakes, and careful review of patients is essential before widespread use of the drug is advocated.

We thank our medical, nursing, and technical colleagues for their help with this study.

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The third author on this paper was Goran B. Klintmalm. Klintmalm was a 29 year old surgeon in the late stages of his training at the Karolinska Institute, Stockholm, when he was sent by Dr. Carl Groth to the University of Colorado in the summer of 1979 for a transplantation fellowship. With his great technical skills, intelligence, and organizational capabilities, he became an important force in the transition from the "old" to the "modern" era of transplantation. As much as anyone else, he and his co-fellow, Shunzaburo Iwatsuki, had one foot in the past and the other in the future. Klintmalm participated in all of the early trials of cyclosporine-steroid therapy for renal and liver recipients in 1979 and 1980 at the University of Colorado. When this program was moved to Pittsburgh in the last days of 1980, Klintmalm became one of the architects for the Pennsylvania program, a role which was repeated years later when he established the multiple organ transplantation program at Baylor University Hospital in Dallas. His liver transplantation program in Dallas is one of the biggest and best in the world.

After acquiring experience with cyclosporine-steroid therapy in cadaveric renal recipients in 1979 and early 1980, 14 liver recipients were entered into a clinical trial at the University of Colorado between March and September, 1980. Two of the patients died on the operating table and never received any immunosuppressive therapy. The other 12 survived operation and 11 (91%) of these lived for at least one year. The remarkable improvement in the outlook with cyclosporine became the single most important factor in the avalanche in clinical activities in liver transplantation that followed in the next 24 months.

The International Transplantation Society met in Boston in 1980 during the 4th of July national holiday. The first results in Colorado with cyclosporine-steroid therapy for renal and hepatic transplant recipients were presented with acceptance by some of the audience and incredulity by most. The claims seemed too good to be true since the maximum follow-up for the liver recipients was only four months, but the optimistic views as well as the recommendations for polypharmaceutical therapy were vindicated in the ensuing months.

Liver transplantation, 1980, with particular reference to Cyclosporin-A

Transplantation Proceedings, 13: 281-5, 1981

T. E. Starzl, S. Iwatsuki, G. Klintmalm, G. P. J. Schröter, R. Weil III, L. J. Koep and K. A. Porter

Thirteen years ago this month, liver replacement with extended patient survival was accomplished for the first time. That little recipient lived for more than a year before dying of metastases from the hepatoma for which she originally had been treated.[1]

Results with conventional immunosuppression

The demonstration of its feasibility did not make orthotopic liver transplantation a widely used clinical procedure, and in fact, only we[2] and Calne and Williams of England[3] have persisted in large scale trials. Two years ago in Rome, we summarized our experience and that of the British group using conventional immunosuppression with azathioprine and prednisone, to which in the Colorado series we had added lymphoid depletion with antilymphocyte globulin (ALG) or, more recently, thoracic duct drainage.

Our results have been so thoroughly reported that I will dwell on them in summary only, and then mainly to emphasize how unsatisfactory they have been. By early summer of 1976, we had treated 111 consecutive patients. Thirty-one (28%) of these recipients had survived for at least a year (Fig. 1). Now, with follow-ups of 4.5-10.5 years, 13 patients are still living. The flatness of the late life survival curve has been an important stimulus to persist in these efforts, and so has the very acceptable quality of life of these chronic survivors. Chronic graft rejection has been the single most common cause of late death.[2]

A second Colorado series of 30 patients was compiled in the subsequent 18 months, ending in early 1978. Half (50%) of these patients survived for at least 1 year, and today, after 2.5 to almost 4 years, 13 (43%) are still living (Fig. 1). It was thought that improvements in surgical technique (especially biliary tract reconstruction), better diagnosis of postoperative hepatic dysfunction, and refinements in immunosuppression were responsible for the better results.

It is distressing to report that we were unable to maintain these gains in a further series of 30 patients, of whom the first 23 have been documented elsewhere.[2] Instead of using ALG, many of these last 30 patients had lymphoid depletion with thoracic duct drainage[4] or lymphaphoresis. All 30 were given azathioprine and prednisone. The projected 1-year survival is only 33% (Fig. 1). Many of the early deaths in the last series were attributable to technical or management errors, as in the past. These misadventures often were not intrinsically lethal but became so because of the need for high-dose steroid therapy.

The preoperative use of thoracic duct drainage (TDD) as a steroid-sparing device which had been shown to be valuable in cadaveric kidney transplantation[5] proved impractical for conditioning of liver recipients.[4] The amount of thoracic duct lymph drained in patients with chronic liver disease was always large and sometimes it was prodigious. In Fig. 2 is shown a progressive increase in the volume of thoracic duct lymph, which rose to nearly a liter an hour during the 2 weeks preceding transplantation at the same time as the cell yield fell. After successful transplantation, the lymph volumes were halved (Fig. 2). This patient had a good result, but two patients died during preparation for transplantation because of our inability to manage fluid exchange of as much as 2 liters/hr.

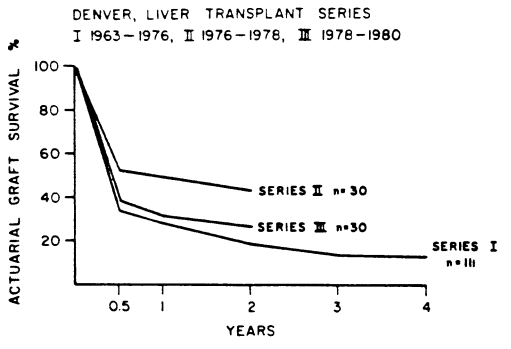

Fig. 1.— Life survival curves of three successive series of patients who were given orthotopic liver homografts at the University of Colorado.

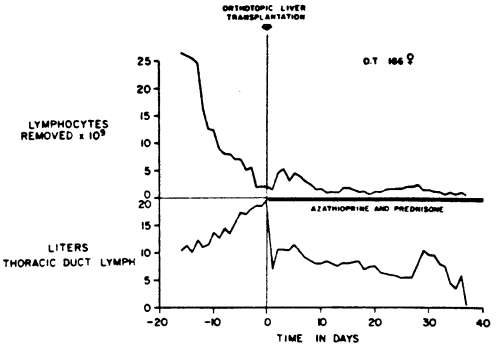

Fig. 2.— Daily lymph volumes and lymphocytes obtained by TDD in an orthotopic liver recipient whose original hepatic disease was primary biliary cirrhosis.

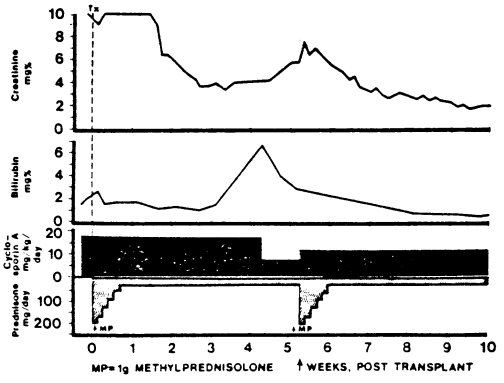

Fig. 3.— The development of jaundice in the recipient of a cadaveric kidney who was being treated with cyclosporin-A. Note decline of the bilirubin after reduction of the cyclosporin-A dose, but with the penalty of renal homograft rejection. Eventually, the combination of prednisone plus an increased dose of cyclosporin-A allowed control of the rejection.

The Justification for Change

By the end of 1979, we had concluded that no real movement of liver transplantation toward an acceptable risk was going to be possible without a drastic change in immunosuppressive techniques. Thus, when the possibility arose of using cyclosporin-A, we had no hesitation in proceeding. As everyone here knows, cyclosporin-A was the product of a Sandoz Corporation research team. The powerful immunosuppressive qualities of cyclosporin-A were accurately delineated in rodents by Borel et al.[6] Calne and his associates of Cambridge, who were the first to use cyclosporin-A in larger animals and humans, reported these trials to this Society in Rome almost 2 years ago.[7] In Calne's most recent comprehensive publication,[8] he described the administration of cyclosporin-A to two liver recipients, of whom both were then alive with follow-ups of a few weeks. By personal communication 2 weeks ago, the number of cyclosporin-A liver cases in Cambridge had increased to five. Three were still alive (longest follow-up 10 months), although one of the three had been switched to azathioprine-prednisone because of nephrotoxicity. The deaths were due to rejection in one instance and an unexplained cardiac arrest 3 weeks postoperatively in the other.

Cyclosporin-A and Renal Transplantation in Colorado

When cyclosporin-A became available for clinical trials in the United States in late 1979, we began its evaluation in the simple kidney transplant model. From the beginning, it was obvious that unless some hidden problem surfaced, cyclosporin-A would change the face of transplantation. Between December 1979 and 1 month ago, we treated 36 recipients of 37 cadaveric kidneys with cyclosporin-A and prednisone. Eleven of the patients also had preoperative lymphoid depletion with thoracic duct drainage (10 examples) or lymphaphoresis (1 example).[9] After 1-6.5 months, 89% of the patients have been liberated from dialysis (Table 1). Two patients died with well functioning kidneys, for a mortality of 5.6%. Two kidneys were lost to rejection, and a third organ was removed because of ureteral necrosis. Even though the follow-ups are still short, the early results (Table 1) have been superior to those achieved by us in the past with any other kind of immunosuppression, particularly considering the fact that retransplantations were included in the statistics.

Thus, we reinforce Calne's optimistic projections about the future role of cyclosporin-A in transplantation. However, our views about how to best use this valuable agent[9] are divergent from those of the Cambridge team, which has warned against combining cyclosporin-A with other agents. In contrast, we have concluded that cylcosporin-A alone, even in doses of 15-20 mg/kg/day, does not consistently prevent rejection, that it should be combined with steroid therapy for optimal use, and that the proper amount of prednisone when cyclosporin-A is used is much smaller than when steroids are combined with azathioprine. Finally, cyclosporin-A has been safely combined with thoracic duct drainage.

Conceptually, we have substituted cyclosporin-A for azathioprine in what is a modern-day version of the time-honored double-drug immunosuppression introduced in 1962 and 1963.[10] Having learned that rejection usually can be expected, we now start the steroids on the day of operation and reduce the prednisone in adults by 20 or 40 mg/day until a maintenance dose of 20 mg/day is reached (usually within 5 or 6 days). The amount of prednisone needed in the first 3 months has been between one-fifth and one-tenth that which we used to give when prednisone was combined with azathioprine.

Besides learning from kidney recipients how to provide immunosuppression, this experience gave insight into the hepatotoxicity that has been seen with cyclosporin-A. We have seen significant hepatotoxicity, including jaundice in 15% of our kidney recipients, always with daily cyclosporin-A doses of about 17.5 mg/kg.[9] This information in kidney graft recipients has been important in making decisions about how to give cyclosporin-A in liver transplant recipients. The patient whose course is shown in Fig. 3 developed jaundice 4 weeks after renal transplantation, while being given 17.5 mg/kg/day of cyclosporin-A. The hepatic dysfunction promptly reversed when the cyclosporin was reduced to 7.3 mg/kg/

Table 1. Cadaveric Renal Transplantation at the University of Colorado Under Cyclosporin-A and Steroid Therapy (Eleven of the 36 Patients Also had Preoperative Lymphoid Depletion With Thoracic Duct Drainage or Lymphapheresis)

	Patients	Grafts	Deaths*	Kidneys Lost Other Than Death	Patients off Dialysis
Primary transplantation	30	30	2	1†	27 (90%)
Retransplantation	6	7	0	2‡	5 (83%)
Total	36	37	2 (5.6%)	3	32 (89%)

*One death from pneumonitis; one death from complication of coronary artery bypass.

†Loss from rejection.

‡One loss from ureteral necrosis, the other from rejection.

Table 2. Orthotopic Liver Transplantation at the University of Colorado Under Cyclosporin-A and Steroid Therapy

OT No.	Age (Years)	Diagnosis	Lymphoid Depletion	Date of Operation	Outcome	mg/dl Bilirubin 7/1/80	mg/kg/Day Cyclosporin-A 7/1/80	mg/Day Prednisone 7/1/80
171	29	Chronic aggressive hepatitis	2 month, TDD	3/9/80	Alive	1.2	12	20
172	24	Hepatoma	No	3/10/80	Alive	4.0	10	25
173	34	Secondary biliary cirrhosis	1.5 month Lymphapheresis	3/21/80	Alive	0.6	11	20
174	20	Budd-Chiari syndrome	No	3/25/80	Alive	0.6	8	10
175	41	Primary biliary cirrhosis	No	4/13/80	Alive	1.8	10	15
176	33	Sclerosing cholangitis	No	5/13/80	Alive	3.0	10.5	20
177	26	Chronic aggressive hepatitis	No	5/17/80	Alive	2.0	10	20
178	37	Secondary biliary cirrhosis	No	5/30/80	Operative death			
179	23	Budd-Chiari syndrome	No	6/5/80	Alive: neurologic damage	3.0	8	10

day. At the new low dose of cyclosporin-A, the kidney graft began to reject, requiring an adjustment of steroids. After weeks of drug juggling, a good result was obtained.

Cyclosporin-A and Liver Transplantation

The first liver transplantation under cyclosporin-A was not attempted until experience had been acquired with 22 cadaveric kidney graftings. Since then, nine orthotopic liver transplantations have been performed (Table 2). There was one operative death, when the abdominal incision could not be closed despite repeated attempts during a 48 hr period. Postoperative therapy with cyclosporin-A was not provided. Of the eight survivors, all are being treated with cyclosporin-A. One has serious residual neurologic injury from a cardiac arrest. The others are well, although not all have perfect liver function. The eight survivors are being treated with 8-12 mg/kg/day of cyclosporin-A plus 10-25 mg/day of prednisone.

We mentioned earlier our conclusions about the optimal way to use cyclosporin-A in kidney graft recipients. It was not surprising that these conclusions seemed to apply in liver transplantation. Five of the eight patients who survived operation were suspected of having rejection within 1-4 months postoperatively. In two cases there were increases in serum transaminases and alkaline phosphatase, but no jaundice. Three other patients also had increases in serum bilirubin.

One to five liver biopsies were obtained in these five patients. In every case the grafts contained mononuclear cells and other findings compatible with cellular rejection. Eosinophiles were more prominent than in rejecting livers under conventional immunosuppression. The liver function abnormalities were promptly ameliorated with steroid therapy, thus reinforcing the histopathologic impression of rejection.

The high incidence of rejection in liver recipients treated with cyclosporin-A but not initially given prednisone has caused us to adopt the same policy of prophylactic steroid immunosuppression (Fig. 4) described earlier for adult renal graft recipients, namely, 200 mg prednisone on the first postoperative day, reduction by decrements of 40 mg/day until 40 mg is reached. On the next day, the maintenance dose of 20 mg is reached from

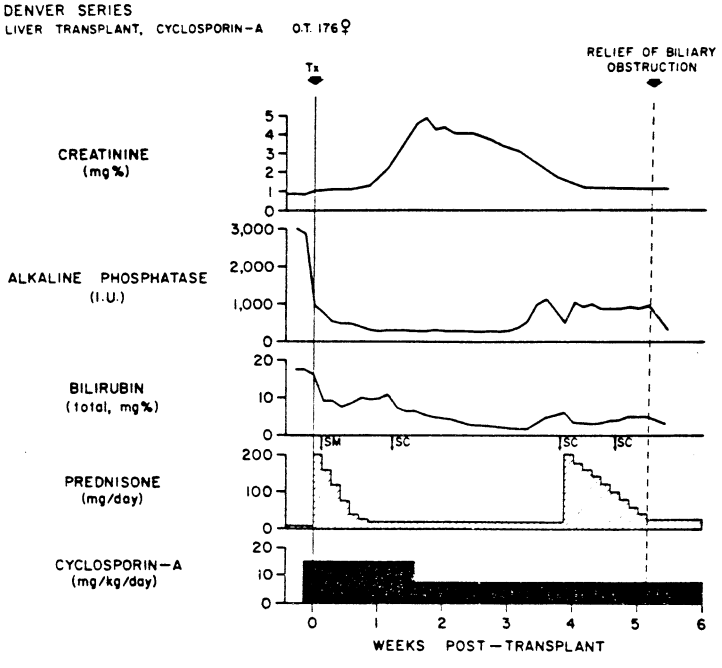

Fig. 4.— The development of uremia in an orthotopic liver recipient treated with cyclosporin-A. Kidney function returned to normal after a reduction in cyclosporin-A dose. Note that prednisone was given from the beginning, but in rapidly decreasing amounts. Later (not shown), the penalty of decreasing immunosuppression was liver homograft rejection as judged by liver biopsy. An increase of the cyclosporin-A dose plus steroid therapy seemed to control the rejection, but subsequently it was found that the liver homograft was partly constructed. The cholecystojejunostomy was converted to a choledochojejunostomy, with amelioration of the low-grade jaundice.

which further slow reductions (or increases) are individualized (Fig. 4). The development of rejection in spite of such treatment signals a need for more steroids. We do not respond by drastically increasing doses of cyclosporin-A, since we have learned from kidney graft recipients that the hepatotoxicity range is entered with daily doses of 15-20 mg/kg. Furthermore, nephrotoxicity could be the price of such adjustments. Three of our eight liver recipients under cyclosporin-A have developed renal dysfunction, which promplty improved after a dose reduction (Fig. 4).

Summary

The field of liver transplantation, which had reached a state of tantalizing but incompletely fulfilled promise, has been revitalized by experience with the new immunosuppressive drug, cyclosporin-A. For optimal value, cyclosporine-A in both kidney and liver recipients has required steroid therapy, but the amounts of prednisone have been a small fraction of those used in the past. It seems to us that the cyclosporin-A-prednisone combination should permit a new chapter to be opened in transplantation.

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III

Part III
The Human Trials:
The First Cases

Part III
The Human Trials

The first cases. By 1 March 1963 when the first clinical attempt at orthotopic liver transplantation was made, the technical problems with the operation were reasonably well understood although not completely resolved. The recipient was a 3 year-old boy with biliary atresia who had had multiple previous operations. The transplantation could not be completed because of hemorrhage from venous collaterals and because of a coagulopathy. Even for a team that had been fully prepared for technical vicissitudes by hundreds of animal operations, the experience was a shock. Two more liver transplantations were carried out in the next four months. In both the operations seemed satisfactory although the recipients died after 22 and 7-1/2 days.

These first three cases were reported in November of 1963.[1] In July and October, 1963, two more attempts were made at the Universty of Colorado.[2] Isolated trials of liver transplantation also were made at the Peter Bent Brigham Hospital in Boston[3] and in France.[4] These first seven liver transplantations and the causes of failure are summarized in Table I. Pessimism settled in worldwide for more than three years. The operation seemed too difficult to allow practical application. The methods of preservation were inadequate for an organ so seemingly sensitive to ischemic damage. Finally, the quality of immunosuppression available was too primitive to permit success.

TABLE 1: THE FIRST TRIALS OF ORTHOTOPIC LIVER TRANSPLANTATION

No.	Location (Ref.)	Age (yr.)	Disease	Survival (Days)	Main Cause of Death
1	Denver (1)	3	Extrahepatic biliary atresia	0	Hemorrhage
2	Denver (1)	48	Hepatocellular cancer, cirrhosis	22	Pulmonary emboli, sepsis
3	Denver (1)	68	Duct cell carcinoma	7-1/2	Sepsis, pulmonary emboli, gastrointestinal bleeding
4	Denver (2)	52	Hepatocellular cancer, cirrhosis	6-1/2	Pulmonary emboli, ? hepatic failure, pulmonary edema
5	Boston (3)	58	Metastatic colon carcinoma	11	Pneumonitis, liver abscesses, hepatic failure
6	Denver (2)	29	Hepatocellular cancer, cirrhosis	23	Sepsis, bile peritonitis, hepatic failure
7	Paris (4)	75	Metastatic colon carcinoma	0	Hemorrhage

By the summer of 1967, these deficiencies had been rectified at least partially, and many long-term survivors had been obtained in the laboratory, some dogs having passed the four year postoperative mark by this time. Better immunosuppression with the so-called triple-drug therapy was available (cf. Part II).

On 23 July 1967, a 1-1/2 year old child with a huge hepatoma was given a new liver and was restored almost immediately from a morbid state to seemingly good health. More cases followed[5] in what were the first examples of successful transplantation of an organ other than the kidney. The lessons learned were generic and within six months the first attempts were made at transplantation of the heart,[6] pancreas[7] and lung.[8]

By the spring of 1969, 25 patients had received orthotopic liver homografts at the University of Colorado, and one more recipient had undergone an orthotopic heterotransplantation from a chimpanzee. These cases and a summary of all previous work were published in a text in late 1969.[9] Meanwhile, Professor Roy Calne at Cambridge University (England) began his first clinical trials in

1968,[10] and a few months later he formed a collaboration with the hepatologist, Professor Roger Williams at King's College Hospital in London that has endured for two decades. The Colorado and Cambridge teams continued their clinical efforts throughout the years, in spite of frequent disappointments and many tragic failures. Although life was prolonged in a number of patients treated in the 1960's, the longest survival of any recipient through 1969 was five years and 11 months. The longest survivor in the world today received her new liver on 22 January, 1970. That little girl, three years old at the time of operation, just has completed her 18th postoperative year, is married to a United States Marine and lives in Okinawa.

Technical Principles. In principle, liver transplantation is exceptionally straightforward. It involves the removal of the diseased native liver and its replacement with the liver of a cadaveric donor in as anatomically a normal way as possible. All of the essential steps were described in the first report[1] including a new technique for intraluminal suturing of vessels that had to be reconstructed without redundancy in close quarters. The technique used by Calne essentially was the same.[10] More complete details of the operation were summarized in the 1969 text.[9] However, great difficulties with some aspects of the operation continued for years.

Bile duct reconstruction. In most of the first human cases, the homograft common bile duct was anastomosed to the recipient common duct over a T-tube stent. Unfortunately, this approach was abandoned in favor of cholecystoduodenostomy, an operation that was somewhat easier technically and one that had worked well in canine experiments. The first inkling that unrecognized biliary obstruction was common came in a report by Martineau et al.[11] Subsequently, the incidence of bile duct complications including obstruction, fistula and cholangitis was shown to be more than 30%.[12,13] Eventually, these problems were minimized with biliary reconstruction by choledochocholedochostomy with a T-tube stent or, if this was not feasible, choledochojejunostomy to a Roux limb.[13,14]

In England, Calne had encountered the same problems with biliary obstruction and fistula,[15] and he adapted for reconstruction an operation developed by Waddell et al[16] at the University of Colorado. With the so-called Waddell-Calne procedure, the common duct of the homograft is anastomosed to the homograft gallbladder that then serves as an extension conduit to the recipient common duct or bowel. The Waddell-Calne operation is not used very often in most centers, but it is a sound procedure that can be useful or even lifesaving in some complicated cases in which an extra length of the homograft common duct is needed.

Veno-venous bypasses. There were reasons why veno-venous bypasses were not used systematically for orthotopic liver transplantation until recently.[14] However persuasive the arguments, the result was that an operation was repetitively performed in humans under conditions that limited its usefulness, increased its perioperative risk and made training of the next generation of hepatic surgeons difficult. The mistake was made of believing that a fundamental principle of surgical physiology worked out in animals, namely, that veno-venous bypass was essential for effective liver transplantation,[17,18] was not truly relevant in humans. The articles defining this principle are reproduced in Part I.

How this error was rectified cannot be traced easily from the articles describing the work. The stimulus for reassessment was a persistent 5-10% intraoperative mortality that was due almost entirely to poor patient tolerance during the venous occlusions of the anhepatic phase. However, nothing decisive was done to rectify the situation until a tragedy occurred in Pittsburgh on 13 May, 1982 that utterly demoralized the transplant team. A teenage hemophiliac male with chronic active hepatitis died on the operating table from the combination of bleeding, third space fluid sequestration and cardiovascular instability that was then common during hepatectomy and the sewing-in of the new liver.

The dimensions of the mother's grief were beyond description and could not be relieved during the long night which members of the transplant team spent with her. The vigil was not made easier by the thought that we might have been hardened by our own repeated failures to the point of no longer making improvements. The program was closed for more than a month until 15 June, 1982 when in the mid-afternoon one of us (TES) went to the office of Dr. Henry T. Bahnson, Chairman of the Department of Surgery at the University of Pittsburgh, for a conversation in which the physiologic

requirements for the veno-venous bypass were discussed. Dr. Bahnson was requested to set up a pump-driven bypass for the next case. Bahnson grasped the essence of the problem instinctively, and he agreed immediately. That night, a liver replacement was carried out under veno-venous bypass in a 6 year old child with biliary atresia. The bypass was performed under 3 mg/kg heparin with a roller pump and other conventional equipment used for open-heart surgery. This technique of a pump-driven bypass had been described in dogs 10 years earlier by Cutropia et al,[19] but their article was unknown to us at the time. There was little trouble in reversing the heparin effect afterwards. All those who were there that night were ecstatic about the ease and non-stressful nature of the transplantation under bypass conditions.

The way in which liver transplantation was facilitated by veno-venous bypass was verified in a number of other cases. By 1 July, 1982 abstracts describing the technique were submitted under the authorship of Bahnson and Starzl to the Southern Surgical Association and to the American Association for the Study of Liver Diseases. Both were rejected. Meanwhile, problems had been encountered with reversal of the heparin effect in several of the adult recipients. Veno-venous bypass under systemic heparinization had worked marvelously in those patients with relatively "simple" diseases such as primary biliary cirrhosis and in recipients who had never had any previous operations. The same was not true in patients with difficult pathology or exceptionally advanced disease and especially in those who had undergone multiple procedures previously. Here, the bleeding from the raw surfaces was so great and the heparin effect was reversed with such difficulty that the value of bypass technique was vitiated. In fact, two patients with veno-venous bypass under heparin died of hemorrhage when clotting could not be restored.

The possibility of reducing or eliminating the systemic heparinization was discussed. From a lifetime of experience with cardiopulmonary bypass techniques, Bahnson was convinced that this would not be safe. However, one of Bahnson's young associates, Dr. Bartley Griffith, thought differently and approached one of us (TES) early in September, 1982 about omitting the systemic heparin. Griffith and Dr. Robert Hardesty, another of Bahnson's associates, had avoided systemic heparin in patients with pulmonary insufficiency who had been supported with extracorporeal membrane oxygenators. Griffith and Hardesty just had purchased an atraumatic centrifugal pump that they thought would permit the pumping of venous blood without anticoagulation. The rest of the bypass equipment, including the cannulas and tubing, was not much different than in the first trials under heparin.

On 30 September 1982, work began on dogs in the laboratory. The project was assigned to Dr. Scot Denmark, the resident on cardiac surgical research. Griffith and Denmark provided the bypass capability. The liver transplantations were performed by members of the transplantation service including the senior fellow, Dr. Byers Shaw, Jr. By the end of 1982, most of the work that was reported by Denmark at the Surgical Forum of the American College of Surgeons in October, 1983 already had been completed.[20] However, clinical trials of the non-heparin bypass were not started, in part because it was difficult to predict which patients really needed bypass. In addition, there still was uneasiness about the possibility of clot formation in bypass tubing and consequent pulmonary emboli. Finally, Shaw, who later became an enthusiastic proponent of the technique,[21] was opposed to its use at this time.

During the Christmas season of 1982 and in January of 1983, three more deaths occurred on the operating table in much the same way as with the earlier hemophiliac patient. As a consequence, a decision was made by Starzl at the end of January, 1983 that veno-venous bypasses must be used for all adult recipients of liver transplants from that time onward. In view of Bahnson's previous trepidation about using non-heparin bypass, Griffith, as an additional safety precaution, added the heparin-bonded (Gott) cannulas for both the outflow and inflow sides of the system,[22] even though this was not an integral part of the animal technique. The problems with bleeding that had been aggravated by systemic heparinization were greatly ameliorated. It was obvious that from that moment onward liver transplantation could be a far more reasonable procedure that would be within the capability of many general and vascular surgeons and which henceforth could be taught to surgeons in training in a systematic way.

The sequence of articles from this work was orderly, but the actual publication schedule was not.

The rejection of Bahnson's two abstracts expunged those beginnings from the record with the exception of a brief notation in a review article.[14] The animal work on non-heparin bypasses by Denmark[20] appeared promptly in the literature. However, the descriptions by Griffith et al[22] of the first clinical application of the new method as well as details of technique were delayed. These were sent to *Surgery, Gynecology & Obstetrics* on 29 July 1983. However, the article was not published until more than 1-1/2 years later.[22] In the meantime, an account of the advantages of veno-venous bypasses in liver transplant recipients was given by Shaw at the American Surgical Association in the spring of 1984 and published the following October in *Annals of Surgery*.[21] Shaw acknowledged Griffith's contribution, but the result of the publication delay was that Griffith's article had become almost permanently "In press."

The generous nature of all those who worked on the project made the question of such detail unimportant for purposes of attributing credit or priority. However, it is worth noting how the collegial interactions between the cardiac and transplant surgeons at the University of Pittsburgh made possible a pooling of ideas and resources. The resolution of the bypass problem began with a personal friendship between Bahnson and one of the editors (TES) that was conducive to the sharing of thoughts and objectives to an extent that might not otherwise have been possible. Griffith's entry into the problem and his advocacy of a non-heparin system was central to eventual success; it required an initiative on his part without which the matter of veno-venous bypass might have been dropped. Not the least factor in the story was Bahnson's encouragement of Griffith to pursue an idea that he, Griffith's Chief, initially did not believe to be sound.

Not all liver transplant surgeons believe that veno-venous bypasses are of overriding importance. Calne and his associates have described the use of veno-arterial bypass, sometimes with an intervening oxygenator. They contend that strain on the heart is relieved thereby.[23] Those who have trained with Calne, including Wall et al,[24] have emphasized that liver transplantation can be performed without this extra step, a position also held by the Denver-Pittsburgh team for many years. However, the practicality of liver transplantation with veno-venous bypass is so much greater compared to that in previous times that the principle of bypass is widely accepted.

Coagulation problems. The presence of the coagulation expert, Dr. Kurt von Kaulla, at the University of Colorado in the 1960's was a key element in the development of the transplantation programs there. Von Kaulla studied the renal[25] and hepatic[26] recipients and characterized the clotting defects in both classes of patients. In the first three liver recipients,[1] he demonstrated clotting factor defects, showed the seriousness of fibrinolysis as well as how to treat this problem and recommended the thromboelastogram to follow the minute-to-minute clotting changes in the operating room in much the same way as is recommended and practiced currently. Other studies showing consumption of clotting factors including platelets within the graft itself[27,28] and the development in some patients of a hypercoagulable state postoperatively completed the picture. Flute et al[29] of Cambridge provided confirmatory data. Although this information was available, it was not acted upon systematically for therapeutic correction until the anesthesiologists at the University of Pittsburgh did so in the early 1980's under the direction of Dr. Yoo Goo Kang. Now cautious correction of coagulation defects is an integral part of liver transplantation, greatly diminishing the hemorrhages of nightmare proportions that were common. The other factor that has greatly ameliorated the intraoperative bleeding problems has been the systematic use of veno-venous bypasses.

Refinement in multiple-organ procurement. The techniques of organ procurement and preservation used clinically came from the laboratory procedures (cf. Part I). However, much further development was required for the procurement of multiple organs from human cadaveric donors that were expected to provide kidneys, hearts, pancreases and other tissues as well as livers. The stimulus for improvement came from the hepatic preservation methods introduced clinically in 1976 and 1977 which made liver storage safe for six or eight hours (cf. Part I). Teams from the University of Colorado began to fly to distant cities, particularly Los Angeles on the West Coast, and as far east as New Haven, Connecticut. Calne began to recruit donors in continental Europe.

At first, the individual organs were skeletonized, and after all of the dissection was completed, the kidneys were removed and cold perfused on the back table. The liver and heart then were

removed simultaneously. The removal of all four organs was a rare event, and the first time the kidneys, liver and heart were removed from a single donor was on 17 April, 1978 during a visit by the University of Colorado team to the University of Minnesota.

It quickly became obvious that *in situ* cooling of organs was going to be necessary if extrarenal organ transplantation were to flourish. During the times when the numbers of liver or heart transplants were small, the annoyance caused for renal transplant surgeons by multiple-organ procurement was relatively minor. As multiple-organ procurement became routine, a major educational effort was required to recruit the cooperation of kidney transplanters. The procedures developed in Denver and Pittsburgh were demonstrated throughout the eastern two-thirds of the United States. At the request of the Surgeon General of the United States, Dr. C. E. Koop, a description of the new operation of multiple-organ procurement was published.[30] Modifications of this procedure have been made for unstable donors or even for donors whose hearts have ceased to beat. In the space of less than five years, multiple-organ procurement, using techniques that are interchangeable not only from city to city but from country to country had become the norm in all parts of the world.

Infection control. Bacterial and fungal contamination from the liver was a special and poorly understood problem in the first clinical trials of liver transplantation.[31] A basis for management was laid by laboratory experiments,[32] and by 1969 the principles of therapy were well delineated.[33]

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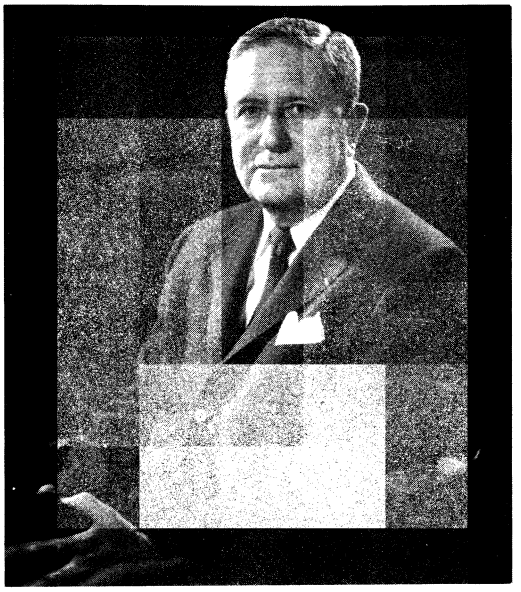
In this account of the first three clinical attempts at orthotopic liver transplantation, the recipient operation was described rather completely, the donor operation and preservation were described in principle in the era before brain-death was accepted or even understood, a relatively complete profile of the clotting changes was provided by von Kaulla and a convincing argument was made for further clinical trials in spite of the deaths of all three recipients.

Efforts at liver replacement had originated in the Northwestern University laboratories five years previously under the sponsorship of the Department Chairman, Dr. Loyal Davis. Davis also was Editor-in-Chief of *Surgery, Gynecology & Obstetrics.* The association helps explain why the report of the first human liver transplants was sent to *Surgery, Gynecology & Obstetrics.* In retrospect, it might have been difficult to find another editor who would publish a paper that was so potentially controversial.

When Loyal Davis died in 1982, one of the editors (TES) attempted to show the Davis influence on the origins of both renal and hepatic transplantation. Excerpts of these reminiscences, from an article published in *Surgery, Gynecology & Obstetrics,** follow:

> In the developments in organ transplantation and hepatic surgical treatment of the last quarter century, Loyal Davis played a peripheral, but by no means insignificant or passive, role. During the first part of this period, Doctor Davis was chairman of the Department of Surgery at Northwestern University, and throughout almost all of it, he was Editor-in-Chief of*Surgery, Gynecology and Obstetrics.* The fact that he functioned in these two powerful administrative positions in an intelligent and creative way was part of the explanation for his long tenure in both offices.
>
> [ ]
>
> Compared with today, grantsmanship in 1958 was a primitive and ingenuous art form. During that year, while still a thoracic surgical resident under Doctor F. John Lewis, I sent a four page grant application to the National Institutes of Health requesting funds of about $30,000 a year for five years. The objectives were to investigate if insulin had different effects if it was given by the portal versus the systemic venous system, to study the effect of endogenous insulin upon the liver and its metabolism and to look at the possibility of ameliorating disorders of insulin and carbohydrate metabolism by portal diversion procedures. The money was awarded.
>
> In retrospect, it was surprising that support was provided. The rationale for the proposal was countercurrent to the prevailing opinion which held that portal venous blood contained no specific substances that distinguished it in any important way from other kinds of venous blood. Complications, such as encephalopathy, were well known to occur after portacaval shunt in dogs or in humans, but these were thought to be the consequences of loss of volume of hepatic blood flow after portal diversion rather than the loss of special portal constituents. The underlying assumption in the grant request was radically different. It suggested that splanchnic venous blood returning from the nonhepatic splanchnic viscera was capable in a special way of influencing hepatic structure and function. In later years, this concept became known as the hepatotrophic hypothesis.
>
> No support could be found in the Northwestern laboratories for the hepatotrophic hypothesis for the simple reason that appropriate experimental models had not yet been developed with which to test the theory. Yet, Doctor Davis always remembered the original question. As the pieces fit into place showing, first, that portal venous blood had specific liver supporting qualities and second, that these qualities were due to hormones (especially insulin) coming from the nonhepatic splanchnic organs, he published the evidence in *Surgery, Gynecology and Obstetrics.* He lived long enough to see hepatotrophic physiology

*Starzl TE: Loyal Davis, Surgery of the liver and transplantation of the kidney. *SGO, 157:* 160-3, 1983



Loyal Davis at the age of 65 years. (1896-1982)

become a defined field of research with numerous applications in clincial medicine (1).

[ ] In order to pursue some of the inquiries which were on my mind in 1958, it was necessary to have a reproducible experimental preparation of total hepatectomy. I developed a method for use in dogs that differed from any previously reported technique in that the retrohepatic vena cava was left intact. The portal system was connected by vascular anastomosis to the vena cava. The procedure has become widely used in laboratory experimentation. In addition, it was realized as a direct consequence of these efforts that it might be possible to replace the extirpated liver with a hepatic homograft. Many of the technical details of total hepatectomy and transplantation were similar, including the use of a venovenous bypass from the lower to the upper half of the body of the animal which allowed temporary decompression of the surgically connected portal and systemic venous beds.

In the summer of 1958, the first efforts at orthotopic hepatic transplantation (hepatic replacement) were made in dogs. The project would have discouraged a more experienced or intelligent investigator, since the first 27 procedures resulted in operative deaths of the recipients. However, the two principles essential for success were finally worked out. One was protection of the venous beds of the intestine, kidneys and hindquarters during the period of venous occlusion as the new liver was inserted; this was accomplished by more efficient veno-venous bypasses. The other principle, effective graft preservation, was met by core-cooling the liver with cold lactated Ringer's solution. This simple preservation technique became standard for laboratory transplantation research with other organs and in clinical renal transplantation.

Once the technical principles essential for success had been defined (2), the operation of hepatic replacement could be studied in detail. Methods could be tested for the prevention of the rejection process which was the next great barrier to be surmounted. In the Chicago series of untreated canine liver recipients, the maximum survival period before death from rejection was 20 and a half days.

Efforts to prevent rejection with total body irradiation of the recipient (or of the graft) were completely unsuccessful. It was not until 1963, after I had moved to the University of Colorado, that prolonged survival periods were achieved using drug therapy with azathioprine. In 1964 and 1965, animals which were treated with this drug or later with antilymphocyte globulin began postoperative lives that lasted more than a decade and were terminated by old age.

At Doctor Davis' suggestion, the results of experimental studies on transplantation of the liver from the Northwestern laboratories were published in *Surgery, Gynecology and*

Obstetrics (2). It could not have been too surprising to him in the autumn of 1963 to receive a report of the first clinical trials with this procedure; he accepted the article for publication by return mail (3). Replacement of the liver has become an increasingly successful way of treating end stage hepatic disease (4, 5).

[ ]

When Doctor Davis died, I had known him for 35 years. After I left Chicago, I wrote or called him several times a year, and until the last two or three years, I always made it a point to meet him at the party given by W.B. Saunders Company at the American College of Surgeons for what for me was an important critique of the past year. As he became old, his attendance there became irregular, but when he did not come, he always wrote and apologized. I did the same. I made no major decision in my professional life without consulting him first. During all this time, I never called him by his first name. It was a matter of respect. To some people who did not know Doctor Davis well, he was a hard and unyielding person. I did not see him that way.

Homotransplantation of the liver in humans

Surgery, Gynecology & Obstetrics, 117: 659-76, 1963

T. E. Starzl, T. L. Marchioro, K. N. von Kaulla, G. Hermann, R. S. Brittain and W. R. Waddell

An ideal treatment for several kinds of liver disease would be removal of the diseased organ and orthotopic replacement with a hepatic homograft. Patients with primary carcinoma of the liver, congenital atresia of the bile ducts, and terminal cirrhosis would all be candidates. The application of such therapy depends, first, upon the employment of a satisfactory operative procedure and, second, upon the use of suitable measures to prevent the immunologic rejection of the graft.

Recently, solutions to these problems have evolved which are at least partially satisfactory. The technical requirements for successful canine hepatic transplantation were defined (9). In addition, a regimen of antirejection therapy was developed in patients receiving renal homografts which resulted in consistent prolonged survival of the foreign tissue (11, 12).

In the present study, the application of these advances to the problem of clinical hepatic homotransplantation in 3 patients will be described. The first attempt resulted in failure at the operating table. The course of the second 2 patients establishes the feasibility of such an operation in humans, despite the fact that death occurred 22 and 7-1/2 days after transplantation from pulmonary emboli.

METHODS

Recipient patients. Patient 1 was a 3 year old white male with congenital biliary atresia (fig. 1A). Physical development had been retarded, preoperative weight being 20 pounds. His general condition was poor, with hepatosplenomegaly, jaundice, and ascites. Total bilirubin was 20.7 milligrams per cent with a conjugated fraction of 16.7 milligrams per cent. Alkaline phosphatase was 12.8 Bodansky units. Serum glutamic-oxalacetic acid transaminase (SGOT) was 160 SF units. The hospital course prior to hepatic homotransplantation on 1 March 1963 was uneventful. On 12 February, the patient underwent thymectomy without complication. For 13 days prior to hepatic transplantation, he was given daily doses of azathioprine of 5 to 6 milligrams per kilogram of body weight.

Patient 2 was a 48 year old Negro male with Laennec's cirrhosis and a primary hepatoma (Fig. 2A), proved by operation at another hospital 8 days previous to the transplantation procedure. The tumor and its multiple satellite nodules involved all 4 anatomic segments of the liver and had a localized attachment to the central tendon of the right hemidiaphragm. Except for the diaphragmatic invasion the neoplasm was confined to the liver. His general health had been excellent until 6 weeks previously, when he was admitted to the hospital with weight loss and symptoms suggestive of a duodenal ulcer. He weighed 140 pounds. Blood analyses, during the 48 hours before transplantation on 5 May 1963, were: bilirubin 3.2 milligrams per cent with 1.75 milligrams per cent direct component; alkaline phosphatase 26.9 Bodansky units; SGOT 315 SF units; total proteins 8.3 grams per cent with 2.7 grams per cent albumin. Blood urea nitrogen was 8 milligrams per cent. Serum electrolytes were normal. Complete blood count was within normal limits. Urine was qualitatively positive for bile. The patient had a low grade fever during the preoperative period, which had been present for several weeks during his antecedent hospitalizations elsewhere.

Patient 3 was a 67 year old white male with progressive jaundice. He had received bilateral supracondylar amputations 15 years previously for occlusive peripheral vascular disease. Exploratory operation was performed on 3 June 1963, and an intrahepatic duct cell carcinoma was found (Fig. 3A) which had obstructed both the right and left main hepatic ducts. Two liver biopsies were performed at this time. One week later, the initial phase of the staged hepatic transplantation, to be described, was performed. It was thought that death of a prospective donor was imminent at this time and that the second stage would follow within a few hours. When the abdomen was opened, a massive bile peritonitis was encountered due to leakage from the previous biopsy sites. The preliminary dissection was carried out after lavage of the peritoneal cavity, and the abdomen was closed without drainage. Recovery of the proposed donor necessitated a delay of 14 days before the next suitable cadaver candidate became available. During this interval, it was known that the patient had continuing biliary soilage of the peritoneal cavity. His condition by the time of the definitive transplantation had deteriorated considerably. Prior to the final operation on 24 June, analyses revealed: bilirubin 20.4 milligrams per cent with 10.4 milligrams per cent direct component: alkaline phosphatase 54.9 Bodansky units; SGOT 110 SF units; fasting blood sugar 100 milligrams per cent. Blood urea nitrogen was 69 milligrams per cent. Urine was qualitatively positive for bile. His general condition prior to the definitive transplantation was poor. He weighed 115 pounds.

Donor patients. The donors were 3, 55, and 69 years old, respec-

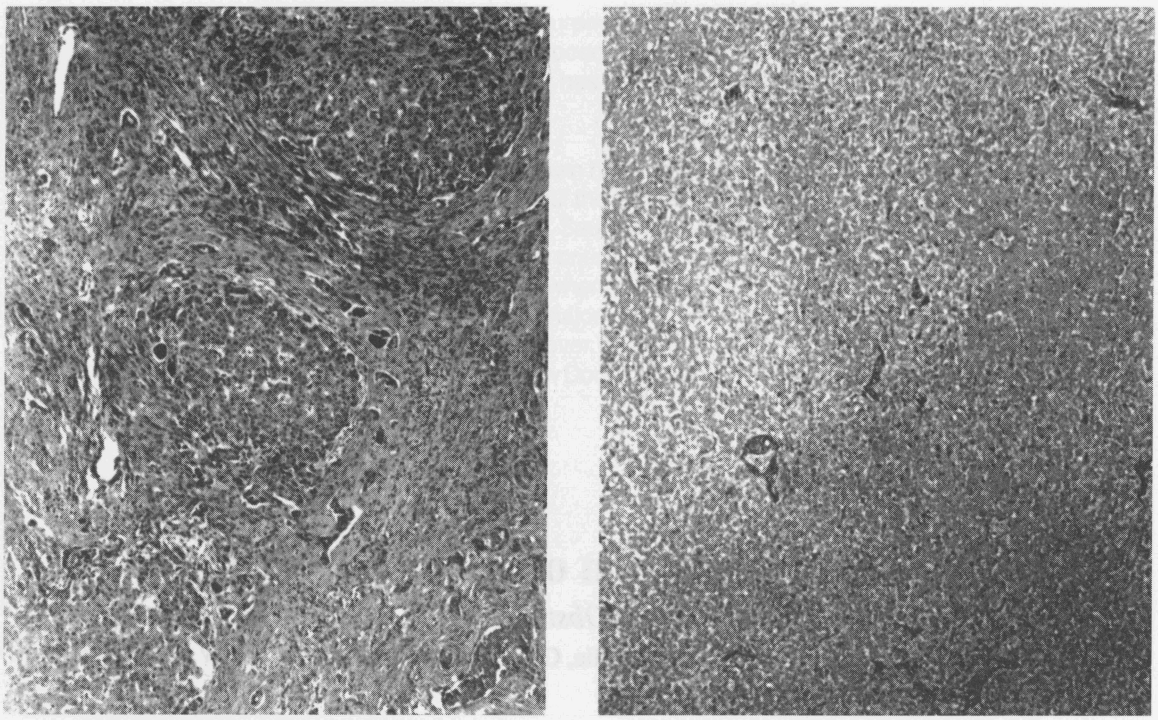


Fig. 1.— Liver tissue in Patient 1. A, left, Patient's own liver, showing advanced biliary cirrhosis. This 3 year old child had congenital atresia of the bile ducts. B, right, Appearance of homotransplanted liver at autopsy, 12 hours after death. The patient exsanguinated on the operating table, 4 hours after revascularization of the homograft. Note extensive autolysis. Hematoxylin and eosin, X19.

tively. The first patient died on the operating table during attempted removal of a third ventricular brain tumor. The second donor died after a protracted terminal illness caused by a cerebral astrocytoma. The third donor died 2 days after massive cerebral hemorrhage.

The circumstances immediately preceding death were different in the first compared with the last 2 cases. The donor for Patient 1 had a cardiac arrest for which open cardiac massage was carried our for 45 minutes before death was acknowledged to have occurred. An additional 15 minutes was required for insertion of the extracorporeal perfusion apparatus. In Donors 2 and 3, respiratory arrest preceded the disappearance of heart action by several minutes. The latter 2 patients maintained blood pressures of 100 millimeters of mercury until a few moments before death. The extracorporeal perfusion was begun 5 and 6 minutes after pronouncement of death.

Donors were selected of the same major blood groups as the recipient patients. In Patients 1 and 2, the blood groups were A+. In the third case; the donor was O- and the recipient O+. Evaluation of liver function in the first donor was not possible. In Donors 2 and 3, complete liver chemistry levels were obtained and found to be essentially normal. The donor liver for Patient 2 was used despite a history of episodic alcoholic excess.

In the last 2 cases, it was possible to maintain a very close vigil on the donors for the 24 to 48 hours preceding their death. Two measures were followed with particular interest, the blood pressure and the hourly urine output. In both cases the maintenance of an effective blood pressure and the continued excretion of urine were considered to be evidence that good tissue perfusion was present until just before death.

Donor operation. Insertion of the catheters and institution of extracorporeal perfusion were accomplished in 15 minutes, 5 minutes, and 6 minutes after pronouncement of death in the donors used for Patients 1, 2, and 3, respectively. The delay in the first case was caused by the necessity to position and prepare the donor's groin. In the 2 subsequent cases, the operative field was prepared and draped prior to death, after approval had been given by the family.

After certification of death, a longitudinal incision was made over the femoral triangle. The femoral artery and vein were cannulated, and the catheters advanced into inferior vena cava and abdominal aorta (Fig. 4). Extracorporeal perfusion was provided with a circuit consisting of a glucose-primed bubble oxygenator, a single DeBakey pump, and a heat exchanger. The priming solution was 2,000 milliliters of 5 per cent dextrose in water, precooled to 15 degrees C. by passage through the heat exchanger. Each liter of perfusate contained 1 gram of procaine hydrochloride, 10,000,000 units of aqueous penicillin, and the amount of heparin calculated to be 1.5 milligrams per kilogram of the donor patient's weight. In Donor 3, 200 milligrams of prednisolone were also added.

Initial flow rates of 30 to 50 milliliters per kilogram per minute were obtained in all 3 cadavers. Cooling proceeded evenly so that body temperatures reached 15 degrees C. 45 to 104 minutes after the onset of perfusion. Flow rates were adjusted to 10 to 20 milliliters per kilogram per minute when the temperature reached 20 degrees C. and flow was continued at this rate thereafter. In Donor 1, venous return began to decline after 2 hours and during the next 110 minutes perfusion ceased altogether. In Donors 2 and 3, reduction in venous return was anticipated and successfully prevented by the addition of whole blood, plasma, and 5 per cent dextrose in water to the oxygenator reservoir. Donor 2 required 4 units of whole blood, 500 milliliters of plasma, and 2 liters of additional priming solution. In Donor 3, 4 units of whole blood, 750 milliliters of plasma, and 6 liters of additional dextrose solution were required to maintain adequate venous return. As will be subsequently described, the low thoracic aorta was clamped in order to concentrate perfusion to the lower half of the corpse.

As soon as it was ascertained that perfusion was adequate, the previously prepared chest and abdomen were entered through a generous right thoracoabdominal incision (Fig. 5A). An incision was immediately made in the fundus of the gallbladder at a site which would be suitable for subsequent cholecystoenterostomy, should this type of biliary anastomosis prove to be desirable. All bile was aspirated to prevent autolysis of the extrahepatic biliary structures. The diaphragm was then incised back to the vertebral column. A vascular clamp was placed on the low thoracic aorta (Fig. 4) to provide selective perfusion of the infradiaphragmatic

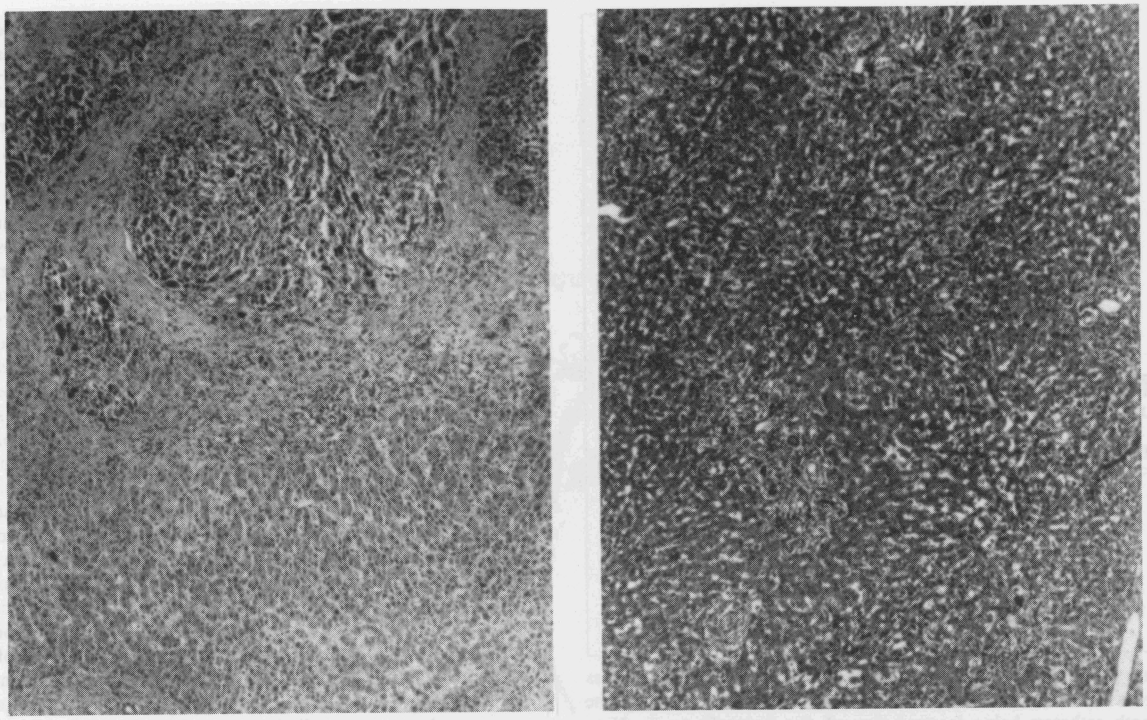


Fig. 2.— Specimens in Patient 2. A, left, Patient's own liver, showing hepatoma. B, right, Liver homograft obtained at autopsy 22 days after operation. Note good preservation of architecture. There was slight periportal fibrosis which is thought to have antedated transplantation. Note mild cholestasis and fatty metamorphosis. A few aggregates of mononuclear cells were present in the periportal areas. Hematoxylin and eosin, X19.

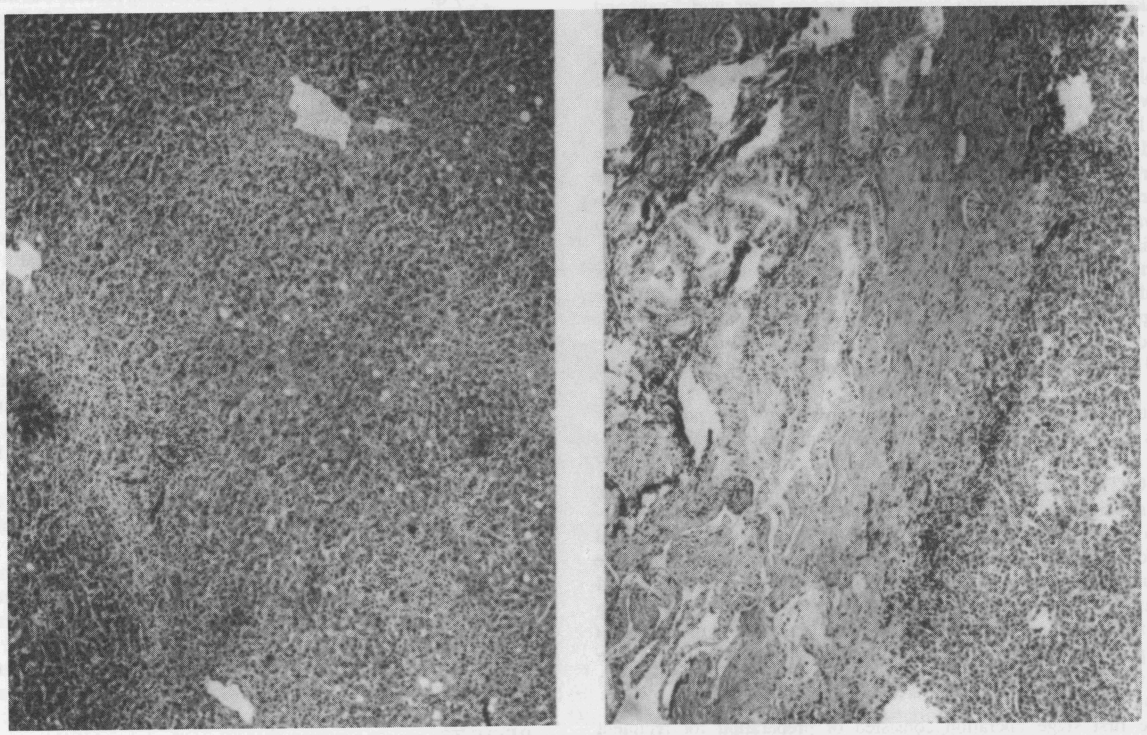


Fig. 3.— Specimens in Patient 3. A, left, Intrahepatic duct cell carcinoma which necessitated operation. B, right, Hepatic homograft 7-1/2 days after transplantation. Note good state of preservation of parenchyma. Periportal accumulations of cells are principally neutrophiles. Hematoxylin and eosin, X19.

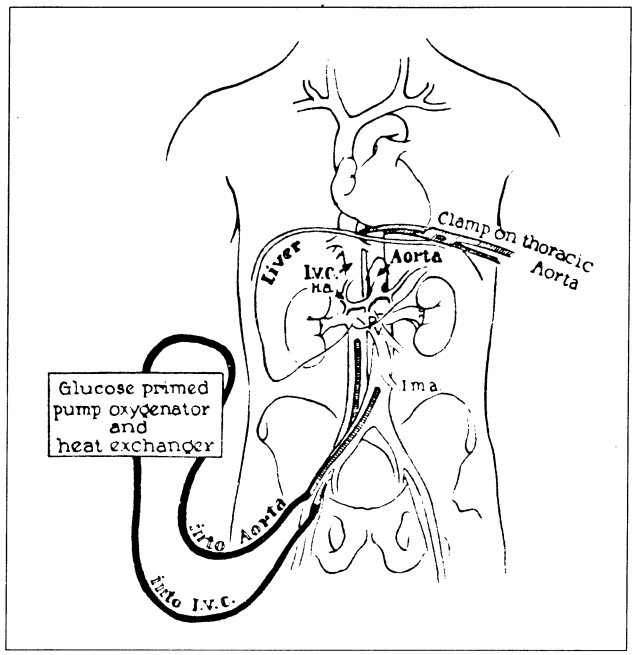


Fig. 4.— Extracorporeal perfusion of the cadaver donor. The venous drainage is from the inferior vena cava and the arterial inflow is through the aorta, both catheters being inserted through the femoral vessels. Note clamp on thoracic aorta to perfuse the lower half of the corpse selectively. A glucose primed pump oxygenator is used with a heat exchanger.

portion of the body. The portal structures were dissected (Fig. 6A), care being taken to obtain suitable lengths of common duct, portal vein and hepatic artery. All extraneous tissue was ligated in continuity before division, including the lesser omentum (Fig. 6A).

Following isolation of the portal structures, the infrahepatic inferior vena cava was cleaned off as far inferiorly as the renal veins. Next the hepatic ligaments were divided (Fig. 5B). The liver was then gently retracted to the left and the right adrenal gland carefully dissected from the posterior surface of the right lobe (Fig. 5C). The adrenal veins entering the cava were ligated and divided (Fig. 5C). At the conclusion of these measures it was possible to pass the finger behind the cava from diaphragm to renal veins without encountering any obstruction.

Finally, the short segment of suprahepatic inferior vena cava was dissected free. After cutting of the fibrous union of the vessel with the diaphragm, it was possible to provide more length by entering an areolar plane and bluntly dissecting the tendinous diaphragm superiorly. A site for eventual transection was selected above the point of entry of the hepatic veins.

After this preparation of the liver for removal, extracorporeal perfusion was discontinued. The portal vein was cannulated as far inferiorly as possible and infusion begun with lactated Ringer's solution cooled to 15 degrees C. The liver was then removed by transection of the previously isolated structures as the infusion continued (Fig. 7).

Recipient operation. In Patient 1, the entire procedure to be described was performed at one operation. In Patients 2 and 3, the surgical steps were carried out in two stages. The time interval between the first and second operations was 22 hours in Patient 2 and 14 days in Patient 3. The long delay in the last patient was due to the unexpected recovery of the patient initially proposed for organ donation. Thoracoabdominal incisions were employed in Patients 1 and 2, and a right paramedian abdominal incision in Patient 3.

The first stage operation consisted of preparation for recipient hepatectomy with dissection and skeletonization of those structures to be subsequently anastomosed to the homograft. Consequently, the steps followed were identical to those described in the donor operation (Figs. 5 and 6A). At the conclusion of this stage, the liver was attached inferiorly only by the three constituents of the portal triad and the inferior vena cava (Fig. 6A) and superiorly by the suprahepatic inferior vena cava. The incision was then closed and the patient returned to the recovery room, with provisions for return to the operating room on a moment's notice.

When it was learned that the donor had died, the recipient patient was taken back to the operating room and anesthetized, prepared, and draped. The previous incision was opened. A siliconized plastic tube was inserted into the inferior vena cava, via the femoral vein, and its upper end inserted into the external or internal jugular vein (Fig. 8A). When arrival of the donor liver was imminent, vascular clamps were placed across the suprahepatic and infrahepatic vena cava, the portal vein, and the hepatic artery. The external bypass was then opened, the residual connecting structures transected (Fig. 6B), and the liver removed. In Patient 2, a second external bypass from the splenic to the external jugular veins was used for portal decompression (Fig. 8A), but this clotted after a few minutes.

Reconstitution of the vena cava at the diaphragm was peformed first with continuous No. 4-0 silk, after lateral fixation with stay sutures. A 2 layer anastomosis was performed posteriorly, with eversion of both layers (Fig. 8B). Next the vena caval anastomosis below the liver was constructed in 1 layer with No. 4-0 continuous silk, again with an intraluminal everting technique for the posterior row. After completion of these anastomoses, normal flow was restored through the vena cava and the external bypass was removed. The hepatic artery and portal vein were then reconstructed with No. 6-0 silk, and thereby first arterial and then portal venous flow was established (Fig. 8C). In Patient 1, biliary drainage was provided by a loop cholecystojejunostomy, after distal ligation of the common duct, this simple method being selected because of the moribund state of the patient. In Patients 2 and 3, a 2 layer choledochocholedochostomy was performed with fine catgut and silk. A T tube was placed in the recipient portion of the common duct, with one limb passing through the anastomotic site (Fig. 8C). Both subphrenic spaces were extensively drained.

Because of the fatal multiple pulmonary emboli in Patient 2, a vena caval plication was inserted in the last patient, midway between the renal veins and the caval bifurcation. Three mattress sutures were used to create 4 small channels.

Coagulation studies. The over-all clotting process was monitored by serial thromboelastograms, as described by von Kaulla (13). These provide continuous mechano-optical recordings of the onset and progress of fibrin formation and fibrinolysis, insight into speed and kinetics of coagulation being afforded thereby as well as information on the final firmness of the clot. The thromboelastograms were supplemented by Quick one stage determinations of the prothrombin complex, plasma fibrinogen analyses by the method of Ratnoff, and measures of the thrombin time by the method of von Kaulla (14).

Fibrinolytic activity was serially measured by the euglobulin lysis time of von Kaulla (15). This method has the advantages of speed and simplicity. In addition, the results are affected neither by heparin nor by the antifibrinolytic drug epsilon-aminocaproic acid (EACA) which was administered to all 3 patients. The technique measures primarily plasminogen activator rather than plasmin (fibrinolysin). Normal lysis times are 120 minutes or longer.

Therapy to prevent rejection. The general scheme of treatment was similar to that previously used for renal homografts (11, 12). In Patient 1, thymectomy was performed 16 days before transplantation. In addition, 5 to 6 milligrams of azathioprine per kilogram were given daily for 13 days preoperatively.

In Patient 2, 4.5 milligrams of azathioprine per kilogram and 30 milligrams of prednisone were given the day preceding definitive transplantation. Splenectomy was performed at the time of the first stage operation. Postoperatively, 1.5 to 6 milligrams of azathioprine per kilogram were given daily, either intravenously or orally. Intermittently actinomycin C was administered intravenously (Fig. 9). Azaserine, 10 milligrams per day, was given intravenously for the first 2 days after operation. A similar regimen was followed for Patient 3, except that neither splenectomy nor thymectomy was performed and azaserine was not used.

RESULTS

Times from death to revascularization of the homografts. The total intervals from donor death to revascularization of the homograft in the new bed were 465, 152, and 192 minutes. Fifteen, 5, and 6 minutes elapsed,

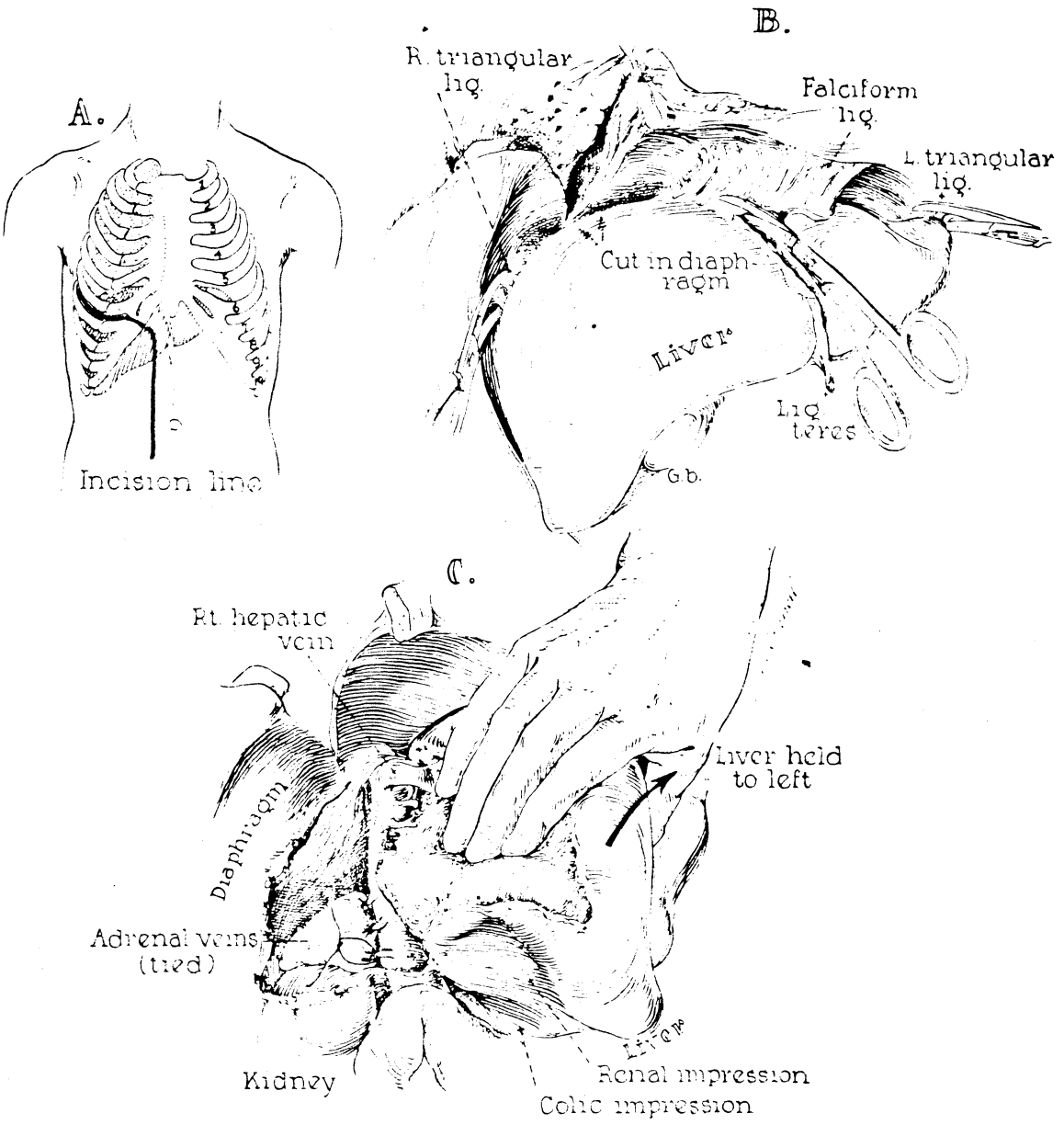


Fig. 5.— Preparation for extirpation of the liver. The steps followed are essentially the same for the donor and recipient operations. A, Line of incision used for all the cadavers and in Patient 2. B, Mobilization of the falciform, triangular, and coronary ligaments. C, Dissection of the right lateral and posterior surfaces of the inferior vena cava; ligation of the adrenal veins. After completion of this maneuver, it is possible to sweep the finger from the diaphragm to the renal veins without meeting resistance.

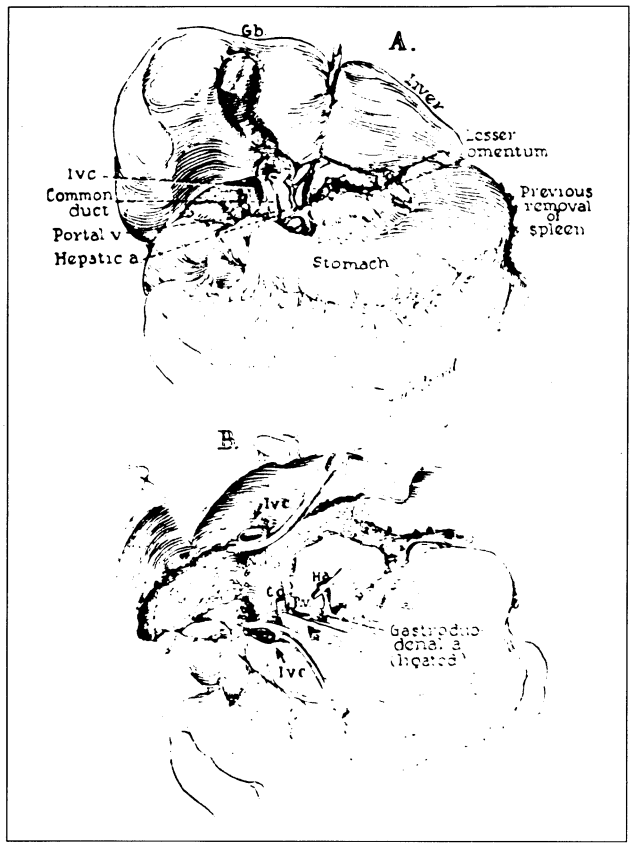


Fig. 6.— Final steps in removal of the liver. A, Dissection of the structures of the portal triad and division of the lesser omentum. B, Operative field after recipient hepatectomy.

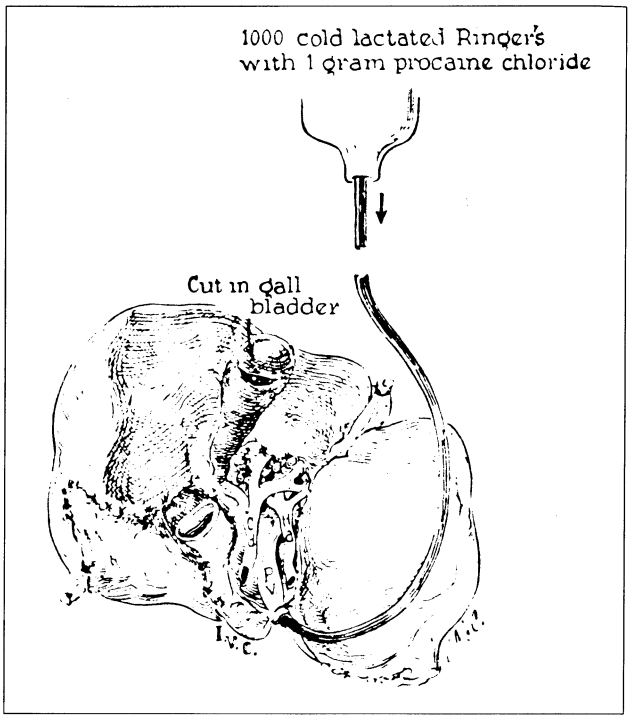


Fig. 7.— Donor liver after removal from cadaver. The blood is washed from the donor organ by gravity perfusion through the portal vein. Note the incision in the gallbladder, employed to prevent autolysis by entrapped bile during harvesting.

respectively, from pronouncement of death to the institution of extracorporeal perfusion. The 3 perfusions, including the brief final infusions, lasted 375, 98, and 126 minutes. The time lapses from removal of the donor liver to rearterialization were 75, 49, and 60 minutes, an additional 10 to 26 minutes being required for the subsequent reconstruction of the portal vein.

Survival. The first patient bled to death on the operating table, 4 hours after revascularization of the homograft. The second and third patients lived for 22 and 7-1/2 days, respectively.

Clinical course. Respiratory insufficiency was the most prominent feature of the postoperative course in the last 2 patients. With Patient 2, air hunger was evident immediately after recovery from anesthesia. At this time, blood sugar was 350 milligrams per cent; serum lactic acid 33 milligrams per cent, normal 6 to 16 milligrams per cent; serum pyruvates 2 milligrams per cent, normal 0.7 to 1.2 milligrams per cent; arterial oxygen tension 54 millimeters of mercury; arterial carbon dioxide tension 25 millimeters of mercury; and plasma pH 7.28. Spontaneous ventilation was 25 liters per minute. The data were interpreted as being consistent with an alveolar-capillary diffusion defect. Eighteen hours postoperatively, tracheostomy was performed, and the patient was maintained on a closed system respirator for the next 30 hours. After withdrawal from the respirator, mild dyspnea persisted, but this was not progressive until the last 48 hours when respirator support again became necessary. Four days after operation, evidence of extensive thrombophlebitis developed in the right lower extremity. Intravenous and intramuscular heparin therapy was provided from the eleventh to the fifteenth postoperative days with decrease in the swelling of the leg. Oral diet was taken from the fourth to the nineteenth postoperative days. Terminally, high fever developed. The roentgenogram of the chest, which had previously been clear, showed the appearance of extensive consolidation in the left lung during the last 3 days of life.

The third patient had normal respiration for the first 72 postoperative hours. Acute dyspnea then developed, necessitating tracheostomy and mechanical ventilation for the rest of his life. One day later, radiographic evidence was noted of extensive consolidation of both lower lung fields. Despite the previous performance of a vena caval plication, pulmonary emboli from the lower extremities were suspected because of the appearance of edema in the leg amputation stumps. Intravenous heparin therapy was instituted from the third to fifth postoperative days and then discontinued because of gastrointestinal hemorrhage. His condition progressively deteriorated, with increasingly difficult ventilation and with continuing gastrointestinal hemorrhage, until his death 7-1/2 days after transplantation. Oral dietary intake could never be resumed.

Alterations in the coagulation mechanisms. Essentially normal preoperative thromboelastograms were obtained in Patient 1, despite a moderate reduction of the prothrombin complex — prothrombin time 53 per cent. After implantation of the liver there was an extreme activation of the fibrinolytic system as demonstrated by almost immediate dissolution of the clot on the thrombelastogram (Fig. 10) and by a euglobulin lysis time of 5 minutes. Intravenous infusion of 0.1 gram of EACA per kilogram abolished completely the fibrinolytic activity (Fig. 10). The clot obtained after EACA administration was, however, structurally poor (Fig. 10) because of the low plasma fibrinogen content of 95 milligrams per cent, and the hemorrhagic diathesis proceeded to a fatal termination. After administration of EACA, the euglobulin lysis time remained unaltered, which indicated that the mechanism causing excessive levels of plasminogen activator substance had not been corrected.

Patient 2 had a normal preoperative prothrombin complex and thromboelastogram. Plasma fibrinogen averaged 400 milligrams per cent, and the euglobulin lysis time was 3 hours. At the first stage operation, mobilization of the liver evoked a marked increase of fibrinolytic activity (Fig. 11). This change returned to normal near the end of the procedure at a time when therapeutic infusions of fibrinogen and fresh blood had been started. None of the other clotting parameters were markedly affected (Fig. 11). During the actual liver transplantation at a second stage the fibrinolytic

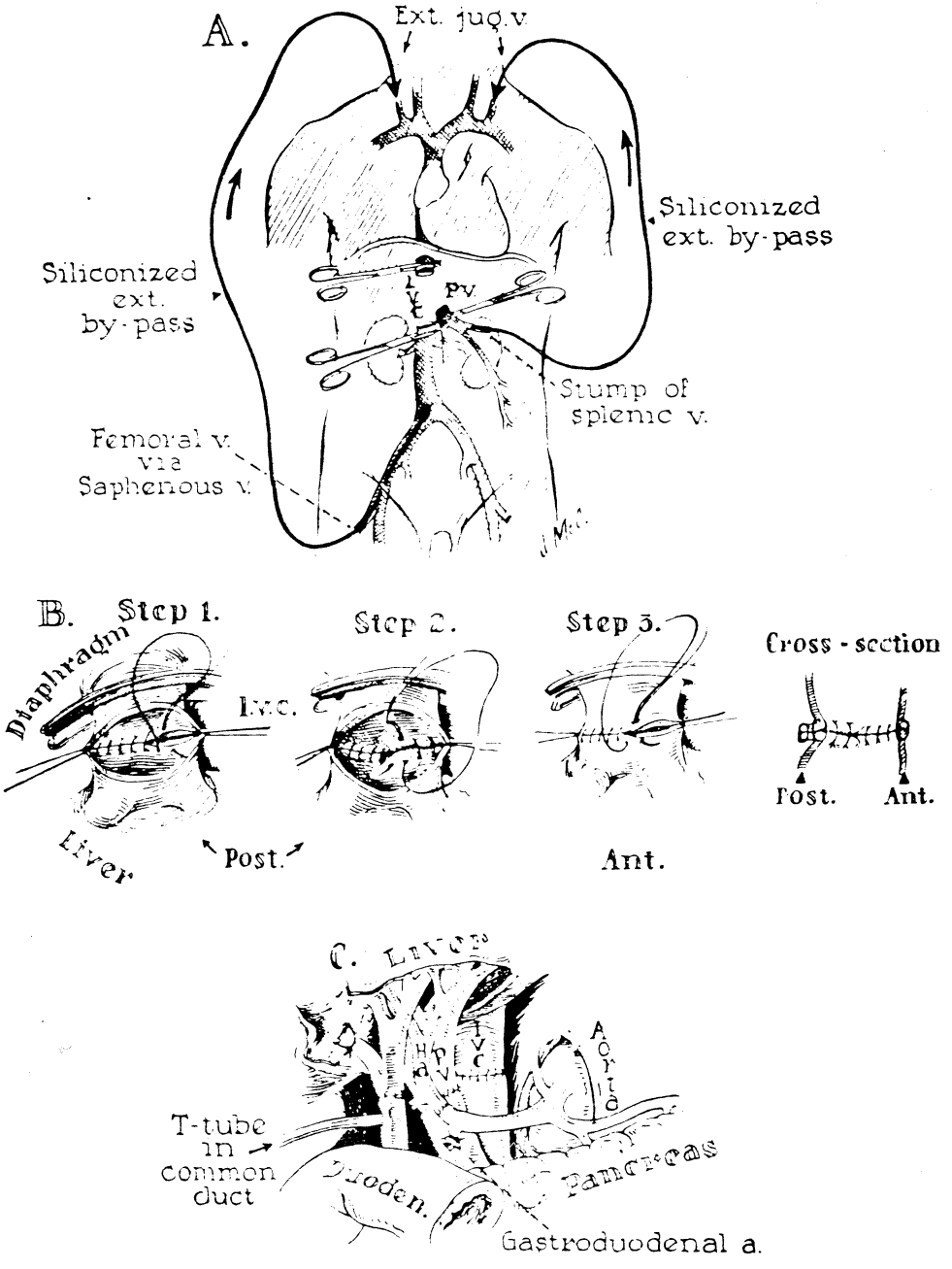


Fig. 8.— Anastomotic procedures in hepatic transplantation. A, External bypasses for decompression of the inferior vena caval and splanchnic venous beds. Both bypasses were inserted into the cervical jugular system. The splanchnic venous catheter used in Patient 2 was inserted after removal of the spleen. Portal decompression was found to be unnecessary providing the caval bypass functions satisfactorily and is of sufficiently large caliber. B. Anastomosis of suprahepatic inferior vena cava. Note that the cuff of the homograft is actually a confluence of the hepatic veins and the vena cava. The posterior row is performed in 2 everting layers. If considerations of time are not pressing, the anterior row is also doubly sutured. C. Subhepatic operative field at completion of all anastomoses. Note that gallbladder has been removed and that the T tube is inserted through a stab wound in the recipient portion of the composite common duct, with the upper limb passing through the anastomosis.

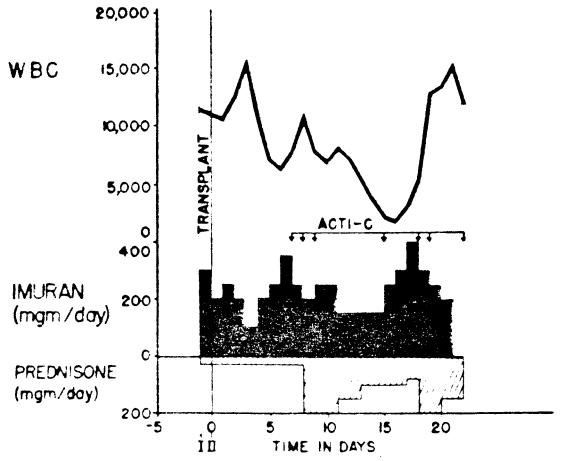


Fig. 9. — Pharmacologic therapy provided to prevent rejection in Patient 2. Avoidance of leukopenia was attempted by careful selection of doses of azathioprine (imuran). *ACTI-C*, Each arrow is 200 micrograms of intravenously administered actinomycin C. *I*, First stage operation; *II*, Second stage operation.

activity increased drastically. Occlusion of the hepatic circulation as the last preparatory step for hepatectomy was followed by a very pronounced shortening of the euglobulin lysis time despite the preceding prophylactic infusion of fresh blood and fibrinogen (Fig. 12). The thromboelastogram showed progressive dissolution of the clot obtained at this time. Twenty-eight minutes after beginning recipient hepatectomy, 0.1 gram of EACA per kilogram was administered intravenously, abolishing the fibrinolytic activity as measured with the thromboelastogram. The euglobulin lysis time was again unaffected and remained pathologically short for more than 2 hours (Fig. 12), which indicated that high levels of plasminogen activator persisted during this period. During the latter part of this interval, the new liver was not immediately corrected with the provision of the homograft. Approximately 1 hour after revascularization of the homograft, the euglobulin lysis time had returned to normal values. There were no clinical signs of bleeding during the operative and the postoperative period at either stage of the procedure. Other clotting measures remained within normal limits except for a moderate rise of the thrombin time during the actual transplantation.

For several days after operation, the thromboelastograms indicated a progressive tendency to hypercoagulability. There was shortening of the "r" values of the thromboelastogram and a concomitant shortening of prothrombin time. Starting on the fifth postoperative day, a progressive fall of plasma fibrinogen level was noted. The euglobulin lysis time was frequently unusually long — many hours. Retrospectively, these later observations are best interpreted as indications of intravascular clotting.

The changes in coagulation in Patient 3 were comparable to those of Patient 2, both quantitatively and qualitatively, except that the shortening of euglobulin lysis time persisted for several hours longer. Therapy with fresh blood, EACA, and fibrinogen was similar to that employed in Patient 2.

Liver function. Serial postoperative liver functions were obtained in the last 2 patients. The presence of hepatic function was evident from the facts that blood sugars could be maintained at normal levels without intravenous glucose, that bile in quantities of 150 to 600 cubic centimeters per day was collected from the T tube the bilirubin content of which was 65 per cent or more conjugated, that no evidence of hepatic coma was observed, and that secondary bleeding did not occur except when heparin therapy was being administered.

There was evidence that moderately severe parenchymal injury occurred at the time of transplantation. The serum glutamic-oxalacetic acid transaminase values rose to 1,150 and 990 units within a few hours in Patients 2 and 3, respectively, but these were returning toward normal in 24 hours (Fig. 13). Lactic acid dehydrogenase and serum glutamic pyruvic acid transaminase values followed similar curves. Bilirubin rose to 12.8

Fig. 10.— Thromboelastograms of Patient 1. A, B, Two hours after revascularization of homograft. Bleeding diathesis was evident clinically. Note onset of clot formation occurs at normal time, since "r" value — time from start of record to clot deposition — is normal. However, the clot is tiny and is lysed as fast as it is formed. C, D, Fifty and 20 per cent of normal fresh blood added to specimen A delays but does not prevent clot lysis. E, Recordings after 0.1 gram per kilogram intravenous EACA. Fibrinolytic activity is abolished. The abnormally small thromboelastogram is due to low fibrinogen. Hemostasis could never be obtained.

milligrams per cent after 9 days in Patient 2 and then progressively improved (Fig. 14). In Patient 3, the bilirubin dropped from 20.4 milligrams per cent preoperatively to 7.2 milligrams per cent after transplantation. During the last 3 days of life, concomitant with the gastrointestinal bleeding, bilirubin again rose, to 15.4 milligrams per cent.

Alkaline phosphatase began to fall immediately after operation in both the second and third patients. The lowest postoperative prothrombin time in Patient 2 was 43 per cent and in Patient 3, 26 per cent. The latter value was obtained just before death. Total proteins were maintained at 5 grams per cent or more, although terminally the albumin dropped to 1.6 grams per cent in Patient 2.

The improvement in hepatic function of Patient 2 continued for the entire course. In Patient 3, there was a secondary deterioration of liver function terminally, coincident with gastrointestinal bleeding.

Autopsy findings. The anastomoses in Patient 1 were all patent. The liver weighed 220 grams, and on cut surface had a prominent lobular pattern. Histologically, the lobules of the transplanted liver were preserved in general outline only. A few scattered clusters of liver cells were partially preserved, but the remainder were severely autolyzed (Fig. 1B). The cytoplasm was smudged and many nuclei were absent. Portal triads were intact. The sinusoids and central veins were dilated.

The homograft in Patient 2 weighed 1,700 grams. Al vascular anastomoses were intact, although there was a thin layer of circumferential adherent thrombus at the portal vein anastomosis. The right iliac vein and terminal inferior vena cava were occluded with old and fresh clot, above which was a 4 by 1.5 centimeter free-floating, nonocclusive thrombus. Multiple large and small emboli occupied both pulmonary arteries. There was massive infarction of the left lung as well as the right lower lobe. Histologic sections of the liver revealed good preservation of lobular architecture (Fig. 2B). There was considerable bile statsis and a mild degree of fatty metamorphosis and periportal fibrosis. A few aggregates of mixed neutrophiles and mononuclear cells were in the periportal areas (Fig. 2B). Some hepatic cells had finely granular cytoplasm with shrunken nuclei.

The transplanted liver in Patient 3 weighed 2,070 grams. The vascular and common duct anastomoses were patent. No thrombi were found anywhere except for 2 small adherent clots just above the site of the vena caval plication. Two pulmonary emboli were found in each lung, 6 to 9 millimeters in diameter. Necrosis of lung had not occurred. There was extensive pulmonary edema. Microscopically, the liver cells were well preserved, although moderate fatty metamorphosis was present (Fig. 3B). Aggregates of periportal cells (Fig. 3B) were chiefly neutrophils.

DISCUSSION

Few experimental studies are available concerning whole organ liver homotransplantation, and all of these involve the use of dogs. Goodrich and his colleagues published the first extensive experiments on hepatic transplantation in 1956. The value of these investigations was limited by the fact that the organs were transplanted to the pelvis, without removal of the dog's own liver. Since then, Moore (4, 5) and Starzl (9, 10) and their

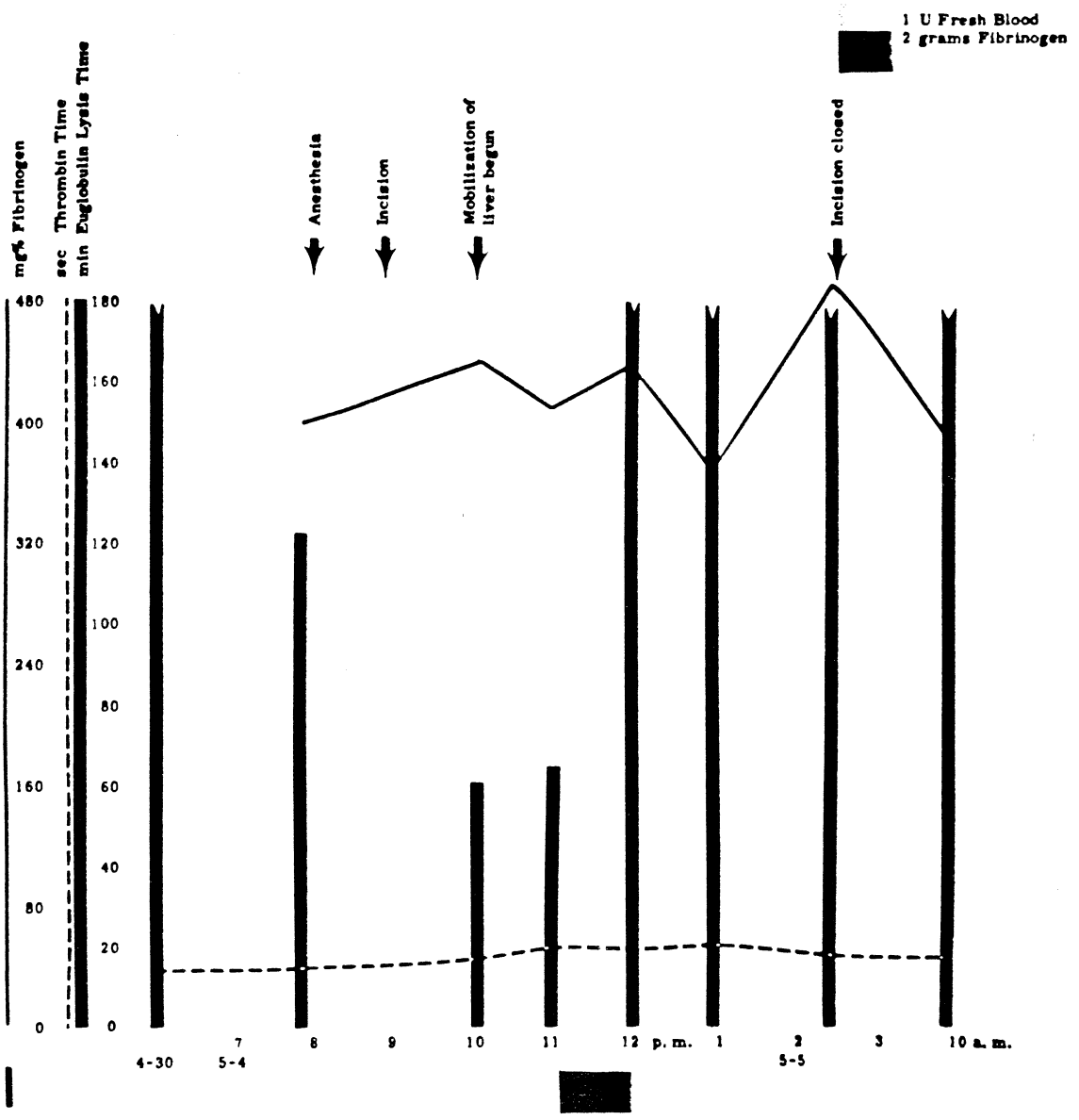


Fig. 11.— Changes in coagulation during first stage operation in Patient 2, when liver was prepared for subsequent removal. Note striking decrease in euglobulin lysis time during mobilization of liver.

colleagues have reported successful hepatic transplantation in dogs with anatomically normal placement of the homograft after removal of the recipient's own liver. These studies clarified the physiological and biochemical events which transpire during rejection of hepatic grafts. In addition, the investigations defined the specific difficulties which must be surmounted if operative failure is to be avoided.

The provision of a viable and minimally damaged homograft is undoubtedly the most important single factor in the determination of success. It is necessary to obtain the donated tissue from a cadaver. Yet the extraordinary sensitivity of the tissue to anoxia requires that an adequate hepatic circulation be present before death and that some form of hepatic preservation be instituted immediately after death before the onset of irreversible cellular injury. For the latter purpose, the hypothermic perfusion technique recently developed by Marchioro and his colleagues was used. It is possible with this method simultaneously to cool and perfuse the liver within a few minutes after death, before beginning its operative removal. The most effective means of postmortem preservation are futile, however, if the moribund state has been protracted. In Patient 1, the selection of a donor after prolonged cardiac massage was unwise. In contrast, the circumstances of death of the donors for Patients 2 and 3 were highly favorable.

Although the technique of hypothermic perfusion has made liver transplantation feasible, the method has definite limitations in extending the postmortem viability of hepatic tissue. Marchioro and his associates have shown that severe hepatocellular injury almost invariably occurs with perfusions of more than 2 hours. The policy of staging the operation in the recipients, which was followed in the last 2 patients, allows reduction of this time to a minimum. After preliminary mobilization of the diseased liver and preparation of the structures for subsequent anastomoses, the definitive second operation can be performed after the incision has been quickly reopened with a minimum of tedious time-consuming dissection.

The surgical details of implantation involve, for the most part, utilization of well standardized surgical methods. The vascular anastomoses frequently must be performed with short cuffs and with limited

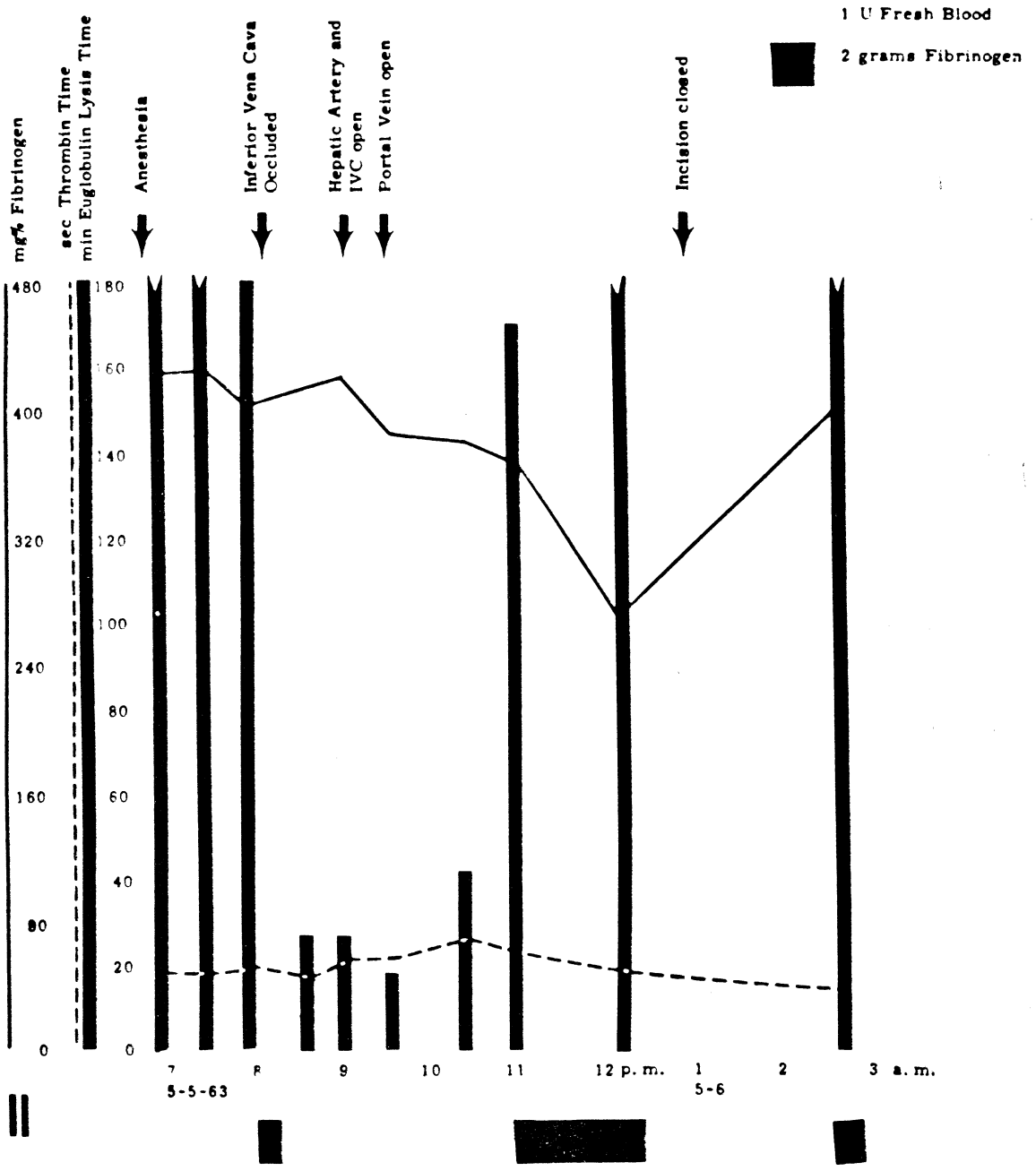


Fig. 12.— Changes in coagulation during definitive transplantation at second stage in Patient 2. Note drastic decrease in euglobulin lysis time and moderate delayed fall in fibrinogen level. EACA, 0.1 gram per kilogram, was given intravenously at 8:47 p.m.

exposure, necessitating intraluminal suturing techniques (Fig. 8B). The greatest hazard is in performance of the inferior vena caval anastomosis at the diaphragm. Both the donor and recipient segments of the vena cava often have orifices of small posterior phrenic tributaries which are unknowingly severed when the respective livers are removed. Such open orifices are located a short distance from the cut edge of and can only be seen from the inside of the vessel. Failure to suture the openings during the caval-caval anastomosis results in later hemorrhage at a time when the presence of the homograft makes secondary exposure of this area almost impossible. The double suture method developed in dogs for this anastomosis has virtually eliminated this problem (Fig. 8).

Reconstitution of internal biliary drainage in infants will probably be most effectively accomplished with a Roux-en-Y cholecystojejunostomy. In the 2 adults, choledochocholedochostomy was simple to perform by the stenting of the anastomosis with a T tube inserted through the recipient portion of the common duct (Fig. 8C). Although the prinicipal arterial supply to the common duct comes from retroduodenal sources, Parke, Michels, and Ghosh have demonstrated that vascular contributions, upon which viability of the donor common duct depends, also come from the hepatic arteries in the hilum of the liver.

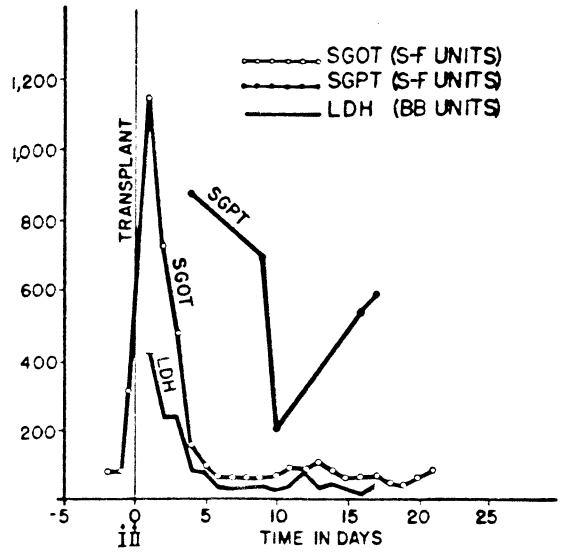


Fig. 13.— Graphic depiction of serum enzyme alterations following hepatic transplantation in Patient 2. Note immediate rise in SGOT with subsequent decline. LDH and SGPT were similarly affected.

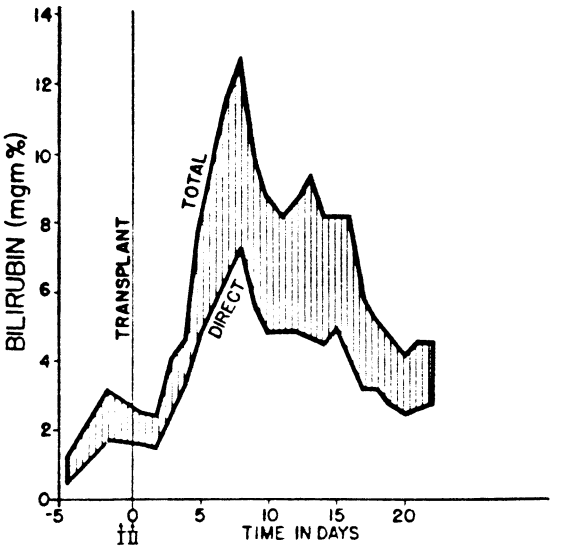


Fig. 14.— Graph showing rise in serum bilirubin to 12.8 milligrams per cent in Patient 2 nine days after transplantation. After transient deepening of jaundice there was progressive improvement.

One unique technical requirement is for decompression of the venous systems which must be temporarily occluded during the transplantation. In the dog, failure to obtain satisfactory drainage from these venous pools results in certain failure, particularly when any degree of venous hypertension is allowed to develop in the splanchnic bed. In man, the necessity for providing drainage from at least the portal system is probably not so important as in the dog. Child has demonstrated that acute occlusion of the portal vein in monkeys and man is usually well tolerated, presumably because collateral channels are more abundant in primates than in lower forms. Indeed, it was demonstrated in the presently reported 3 cases that drainage of the portal system was not necessary. In 2 of the 3 patients, external bypasses were not used, and in the third, flow ceased after a few minutes. The conclusion seems justified that a single effective bypass from the inferior vena cava to the superior vena cava is all that is required for adequate venous return to the heart from both the inferior vena caval and portal systems.

Experience with these 3 patients stresses the need for close control of the coagulation processes during and after transplantation. Profound clotting defects were demonstrated in all at the time of transplantation resulting in fatal hemorrhage in the first patient. The hemorrhagic tendency did not result from an acute deficiency of those clotting factors which are synthesized in the liver. Analysis of plasma fibrinogen during the liverless phase in Patients 2 and 3 did not reveal a clinically significant drop, a finding in accord with many experimental studies which show that a substantial decrease in clotting factors after total hepatectomy requires several hours to develop. Instead, the important finding appeared to be an explosive increase in the plasma fibrinolytic activity which developed within minutes, both during manipulation of the liver (Fig. 11) and after its removal (Fig. 12). The exact cause of this change is open to some speculation. As a working hypothesis, it might be assumed that the liver normally elaborates a substance which inhibits conversion of plasminogen to plasmin (fibrinolysin) presumably by a deterrent action on a plasminogen activator. Absence of the liver or severe hepatic injury during operative manipulation could conceivably permit uncontrolled conversion of plasminogen to plasmin by removal of such a restraining influence on the activator system.

Whatever the explanation for the increased fibrinolytic activity, it is imperative to anticipate this tendency and to provide prophylactic treatment before a frank hemorrhagic diathesis develops. In the second and third patients, EACA was administered prophylactically, within a few minutes after removal of the recipient's liver. This drug, which prevents activation of plasminogen to plasmin by inhibiting plasminogen activators, apparently prevented the fibrinolytic crisis in these last 2 patients despite profound decreases in euglobulin lysis time. The administration of fibrinogen and fresh blood at the time of transplantation are probably also of significant value in reducing the danger of a hemorrhagic diathesis.

In view of the effort expended to define and prevent the hemorrhagic diathesis which led to the death of the first patient, it is ironic that Patients 2 and 3 should have died because of the late consequences of intravascular clotting. In both the latter patients, the immediate cause of death was multiple pulmonary emboli. In both, the characteristic clotting deficiency which was observed at the time of operation was succeeded by a hypercoagulable state several days later, which may have been an important contributing factor in the formation and propagation of the emboli.

The source of the pulmonary emboli in Patients 2 and 3 is a matter of interest. It is encouraging that the vascular suture lines of the transplant itself did not have a selective tendency to develop thrombosis. Rather, the peripheral thrombosis in Patient 2 was in the right iliac vein and the terminal inferior vena cava, which led to the belief that protection from this complication would be afforded by the use of Spencer's vena caval plication procedure. In Patient 3, however, in which vena caval plication was performed, a peripheral focus of thrombus could not be found at autopsy. It is possible in this patient and in the second one as well that semifluid clots were originally formed in and passed through the external bypass system from the inferior to the superior vena cava during the transplantation. The early development of respiratory distress in both patients is compatible with such a sequence of events. In future attempts at hepatic homotransplantation, it is probable that these embolic complications can be prevented with well timed and accurately controlled heparin therapy, either at the time when the external bypass is in use or in the postoperative period.

This experience with humans has confirmed many previous experimental impressions of the functional behavior of the hepatic homograft. In dogs receiving liver homografts under optimal circumstances of cooling and minimal donor organ ischemia, there is a prompt resumption of hepatic metabolism with minimal immediate derangement of function. Biochemical evidence of graft repudiation begins on the fourth or fifth day in the untreated animal, but this can be mitigated or prevented by the use of a therapeutic regimen similar to that employed in the clinical cases. The most useful measurements to follow the course of the rejection process are serum bilirubin, serum alkaline phosphatase, and serum glutamic oxalacetic acid transaminase. Under more adverse experimental conditions, simulating those necessary in a clinical setting, Marchioro and his colleagues have shown that there is a moderately severe ischemic injury to the canine liver, manifested by sharp rises in bilirubin, alkaline phsosphatase

and SGOT within the first 24 hours. Differentiation of the latter nonspecific changes from those due to rejection is an important aspect of the postoperative care.

Both of the patients who survived the operative procedure exhbited a functional pattern of acute parenchymal injury. Rises in SGOT to 950 to 1150 SF units occurred within the first day and then rapidly receded. Jaundice temporarily deepened. The subsequent curves of various function tests were in the direction of improvement until immediately before death, demonstrating the reversibility of these early changes.

The experiences in these cases have demonstrated that the immediate problems of clinical hepatic homotransplantation are subject to practical solution. They provide little information, however, concerning the feasibility of long term maintenance of liver homografts, although the progressive improvement of liver function and the degree of histologic preservation of the transplants after 7-1/2 and 22 days are encouraging notations. In unpublished observations from our laboratories, it has been found possible to obtain prolongation of survival of pharmacologically altered dogs with liver homografts, which has been comparable to that obtained in treated animals receiving renal homografts. Ultimately, it may be necessary to conclude that the treatment required to prevent rejection is significantly different with livers than with kidneys. At present, however, there is no evidence to support such a belief, and the further acquisition of experience in the treatment of these otherwise doomed patients appears to be justified.

SUMMARY

A number of problems are described which must be surmounted for the clinical use of liver homotransplantation, based upon experience with 3 patients. The first patient died of hemorrhage during conclusion of the operation. The second and third patients liver for 22 and 7-1/2 days, respectively, both ultimately dying from multiple pulmonary emboli.

The operative requirements for successful liver transplantation appear to be subject to practical solution. Of the utmost importance is the procurement of a viable and relatively undamaged donor organ. This has been accomplished with the use of an extracorporeal circuit which perfuses and cools the liver immediately after death. In addition, the time interval between death of the donor and restoration of a hepatic blood supply in the transplanted site has been shortened by operating on the recipient patient in 2 stages. At the preliminary operation, all structures are skeletonized above and below the liver with facilitation of the recipient hepatectomy and multiple anastomoses which are performed at the second and definitive procedure. While the transplantation is being performed, the venous return from the splanchnic and inferior vena caval systems is temporarily occluded. It has been found necessary to decompress only the inferior vena cava during this time with an external bypass from the inferior to the superior vena caval systems.

Changes in the coagulation mechanisms constitute a serious deterrent to success. During operation, a bleeding diathesis is regularly detectable by laboratory examination. Postoperatively, a state of hypercoagulability has developed, which probably contributed to the lethal complication of multiple pulmonary embolization in 2 patients. It is also possible that the use of the external bypass contributed to the formation of the emboli.

After operation, hepatic functions were immediately deranged, probably as the result of injury incurred during the transplantation, with progressive improvement thereafter. Later, biochemical evidence of homograft rejection was not observed, and at autopsy in the last 2 patients there was surprisingly good gross and histologic preservation of graft structure. It is thought that the therapy with azathioprine, prednisone, and actinomycin C had forestalled the rejection process.

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In April, 1968 at the Amercian Surgical Association, the first public report was given of the prolonged survival of several patients after liver replacement. The discussion by Frances D. Moore was particularly supportive and has been reproduced.

Orthotopic homotransplantation of the human liver

Annals of Surgery, 168: 392-415, 1968

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Until last year, the kidney was the only organ which had been transplanted with subsequent significant prolongation of life. There had been nine reported attempts at orthotopic liver transplantation; seven in Denver[19,22,23] and one each in Boston[16] and Paris.[5] Two of these patients had succumbed within a few hours after operation,[5,22] and none had lived for longer than 23 days.

This dismal picture has changed within the last 9 months, inasmuch as seven consecutive patients treated with orthotopic liver transplantation from July 23, 1967 to March 17, 1968 all passed through this previously lethal operative and postoperative period. Three of the recipients are still alive after 9, 2-1/3, and 1 months; the others died after 2, 3-1/2, 4-1/3, and 6 months.

Methods

The Recipients. Summary information for the seven patients is given in Table 1. Their ages were 13 months to 16 years. Six were females. The indications for transplantation, which had been established by earlier explorations at other hospitals, were extrahepatic biliary atresia in five cases and liver cell carcinoma in the other two. The clinical condition of the recipients ranged from fair to moribund. The sickest was Patient 5 who had required repeated paracenteses for several weeks before admission and who, as a complication of this procedure, had developed a continuously draining lower abdominal peritoneal fistula that did not close until a week after transplantation. When the patient arrived in Denver, examination revealed renal failure with oliguria, proteinuria, and a blood urea nitrogen (BUN) of 70 mg./100 ml. A few hours before operation massive gastrointestinal hemorrhage developed, probably from esophageal varices. The bleeding continued until after the transplantation had been completed.

This child and the other four with extrahepatic biliary atresia had preoperative serum bilirubin concentration of 16.1 to 30.9 mg./100 ml. and alkaline phosphatase levels of 88 to 632 international units (normal 50 to 150). Other biochemical measures of hepatic metabolism were typical of this disease[14,18] with retention of moderately good synthetic function. For example, the prothrombin values ranged from 50 to 100% of normal and the total serum proteins were 6.5 to 7.9 gm./100 ml. The patients had ascites and all 5 had some evidence of pulmonary arteriovenous shunting as described in a previous publication.[20] None had other congenital anomalies.

In Cases 1 and 7, hepatic cell carcinoma was diagnosed 6 months before transplantation. Although both patients were given liver irradiation (975 and 3,200 R, respectively) and chemotherapy, the tumors had continued to enlarge (Fig. 1). When first seen at our institutions, both children had leukopenia (2,000 and 3,500 WBC/mm.^{3}). They had no evidence of extrahepatic metastases. Liver function was normal.

The Donors. The cadaveric donors were pronounced dead on the basis of cardiac arrest, by members of the service in charge of their care. Previously, all had irreversible central nervous system injury, with no spontaneous respirations and with isoelectric electroencephalograms. In each, life had been maintained for days or weeks with mechanical ventilators and in all but one there had been repeated previous cardiac arrests. After death, ventilatory support and cardiac massage were continued insofar as possible in Cases 1-6 until the organ could be cooled as described below. In Case 7, the adult donor was placed on cardiac pulmonary bypass 10 minutes after death. This technic[15] accomplishes both perfusion and cooling of the cadaver at the same time as hepatectomy is being carried out.

The donors and recipients had variable discrepancies of age, size, and weight. The lingering terminal course of the donors made it possible to chose recipients on the basis of histocompatibility analysis in addition to compatibility of red blood cell types (Table 1). In each case the donor lymphocyte antigens were determined both in our laboratories and by Dr. Paul Terasaki of Los Angeles. The histocompatibility profile was used to select the best of several previously studied prospective recipients. The matches eventually accepted are shown in Table 1. None were perfect, although in Case 1 there were no breeches of 7 major groups of the recently classified human histocompatibility (HLA) system[4,26]; one or 2 group incompatibilities were present in each of the other donor-recipient combinations.

Donor Hepatectomy. Warm ischemia time was minimized by the postmortem resuscitative efforts described above. In addition the livers were core cooled either by the technic of extracorporeal hypothermic perfusion mentioned earlier (Case 7) or by the immediate infusion (Cases 1-6) of cold (2° C.) balanced electrolyte solution containing heparin,

Fig. 1.— Growth of a hepatoma in Patient 1. A — Liver scan in February, 1967, using Au^{198}. The child was then 13 months old. Most of the tumor was in the left lobe but smaller growths in the right lobe precluded resection. B — A repeat study 3 months later showing only a small remnant of functional liver tissue. The excised liver weighed 1,185 grams.

procaine, and low molecular weight dextran, through a cannula inserted into the superior mesenteric vein (Fig. 2). With the latter method, egress for the fluid was provided by incising the inferor vena cava. In order to prevent autolysis the gallbladder was opened at its dome and irrigated with saline to remove all bile.

The aorta was removed in continuity with the hepatic artery (or arteries). It was rapidly exposed and mobilized by lifting the spleen and left transverse colon into the wound (Fig. 3), opening the plane between the pancreas and left kidney, dividing the left renal vein, incising the crura of the diaphragm, and ligating and dividing all branches except the superior mesenteric artery and the celiac axis. The superior mesenteric artery was spared until it could be proved that it did not give rise to part of the hepatic arterial supply.

The rest of the procedure was essentially the same as the originally described[22] except that the cannulated superior mesenteric vein was retained with the specimen; this necessitated ligation of the splenic vein and several other tributaries beneath the neck of the pancreas. In 2 cases with anomalies, a small right hepatic artery was found directly posterior to the portal vein and traced back to its origin from the superior mesenteric artery. The latter vessel was ligated distal to this point (Fig. 4C).

During the final stages of excision, a cannula was inserted into the distal aorta and used to flush the arterial tree with balanced electrolyte solution. In all but Case 2, the homografts were then inserted into a preservation unit for one to several hours. The preservation was with the combined technics of hypothermia, hyperbaric oxygenation, and low flow perfusion of both portal and arterial systems with diluted homologous blood of a compatible red cell type. The total flow rates (6 ml./Gm. liver/hour), temperature (4° C.), times of chamber decompression, and other methodologic details were exactly the same as reported earlier in dog experiments.[2] When the specimens were subsequently removed from the chamber, the vessels were recut according to the requirements for their anastomoses.

Recipient Operation. In six of the seven patients, the operation was not begun until the homograft was in the chamber and had been checked for defects under conditions of perfusion. A large transverse abdominal incision was used. The most difficult step was excision of the recipients' diseased livers since extensive upper adbominal adhesions and portal hypertension were present. In the patients with biliary atresia, the removed organs weighed 665 to 885 grams. The liver from the 19-month-old child with hepatoma weighed 1,185 Gm.; the liver and hepatoma in the 16-year-old girl weighed 2,575 grams. In all but Case 7, the enlarged spleens were removed after the homografts had been placed.

The transplantations were performed with the previously described technic,[22] with the exception of some important details. Biliary drainage was provided by cholecystoduodenostomy (Fig. 4) instead of choledochocholedochostomy. Another extremely significant change, as recently reported,[20] was the omission of bypasses to decompress the splanchnic and systemic venous systems during the anhepatic phase. In the seven patients, the portal vein and inferior vena cava were simultaneously occluded for 50 to 90 minutes. The maximum resulting fall in arterial blood pressure was 20 mm. Hg. The intestines became slightly dusky in several cases, but this change was immediately reversed with the restoration of venous return. The order of the anastomoses was suprahepatic vena caval, infrahepatic vena caval, hepatic arterial, and portal venous. In Case 1, the caval clamps were released after the first 2 anastomoses were completed. In the others, no vascular channels were reopened until at least three, or in two cases, all four vessels had been reconstructed.

In five patients, the homografts hepatic artery or celiac axis was anastomosed to the recipient proper or common hepatic artery (Fig. 4A). In the other two, a separate branch to the right lobe rose from the superior mesenteric artery. The first time the anomaly was encountered (Case 2), the two vessels were individually connected to the right and left branches of the recipient proper hepatic artery (Fig. 4B). The next time (Case 5), the

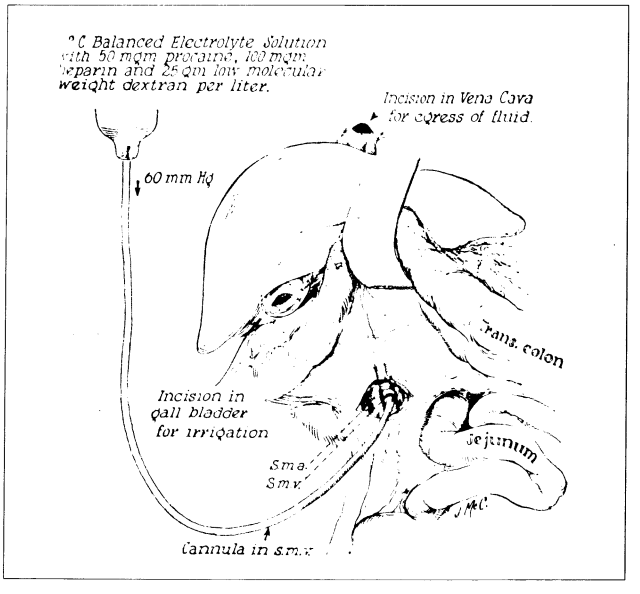

Fig. 2.— Core cooling of cadaveric liver used for infant donors. Immediately after entering the abdomen, the cannula is placed into the readily accessible superior mesenteric vein. This vessel is far enough away from the portal triad so that the portal vein, which will ultimately be used for anastomosis, is not in danger of injury. Egress of the perfusion fluid is provided by the venotomy in the suprahepatic inferior vena cava. Bile is washed from the gallbladder through the cholecystotomy. For the adult donor (Case 7), the liver was cooled by total body extracorporeal perfusion, and the flushing carried out after completion of the hepatectomy.

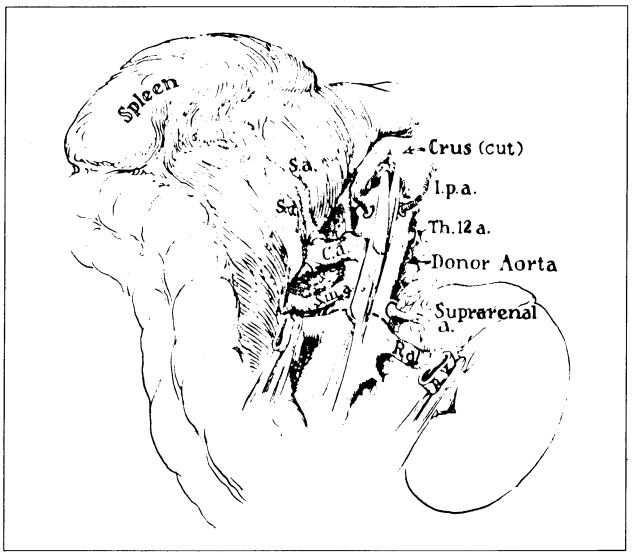

Fig. 3.— Mobilization of the aorta during donor hepatectomy. Rapid identification and ligation of the branches is facilitated if variable traction is applied to the distal aorta. During the initial dissection, all of the aortic branches except the coeliac axis and superior mesenteric artery are ligated and divided; the latter vessel is cut only after it has been shown not to give rise to an anomalous hepatic arterial branch.

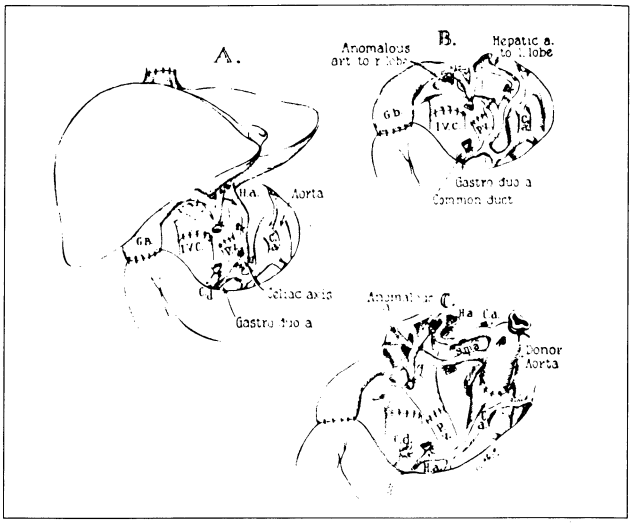

Fig. 4.— Recipient operations. Cholecystoduodenostomy was performed in all 7 cases. A — The kind of arterial anastomosis used in Cases 1, 3, 4, 6, and 7. The homograft coeliac axis or common hepatic artery was attached to the proper or common hepatic artery. B — Arterial anastomoses in Case 2. The right hepatic originated from the superior mesenteric artery. C — Anastomosis of the homograft aorta to the recipient aorta (Case 5). This technic was used because of the double arterial supply.

distal donor aorta was anastomosed end-to-side to the upper abdominal aorta of the recipient (Fig. 4C). Liberation of sufficient recipient aorta above the celiac axis required incision of the covering diaphragmatic crus. The anterior aortic wall was isolated with a partial occlusion clamp during the period of suturing. The proximal end of the homograft aorta was ligated a few millimeters beyond the origin of the celiac axis (Fig. 4C).

As will be mentioned later, Patients 1-3 had temporary paralysis of the right diaphragm. This was thought to have resulted from crushing of the right phrenic nerve by the vascular clamp which was applied to the suprahepatic inferior vena cava. In Cases 4-7, a longer cuff was developed and care was taken not to include any diaphragm in the bite. The complication was avoided.

Intraoperative and Postoperative Management. Monitoring of metabolic changes was facilitated during operation by the use of a sampling catheter in the radial artery. A continuous intravenous infusion of glucose solution was given at 0.5 to 1 Gm./Kg. per hour during the anhepatic phase. Glucose administration was continued postoperatively. When it was inadvertently stopped in one patient for approximately 20 minutes after return to the recovery room, hypoglycemic convulsions resulted. Within 2 or 3 days there was no longer need for continuous glucose. By this time, all the patients had started eating.

Rapidly developing metabolic acidosis was always seen during the anhepatic interval. Data on these changes in the first four cases has been published[20] and the findings in the next three were similar. The extent of the acidosis could not be completely characterized since it was intermittently corrected. In the six infants, sodium bicarbonate was infused during the operation in total doses of 27 to 78 mEq., as guided by frequent determinations of arterial pH, CO2, and bicarbonate. The 16-year-old girl who weighed 39 Kg. received 290 milliequivalents.

Before, during, and for several hours after transplantation, blood coagulation was followed with 11 different tests. These provided a measure of fibrinolysins, platelets, six clotting factors produced by the liver, and Factor VIII (antihemaphilic globulin) which is extrahepatic in origin. These results are being reported separately in detail.[9] Epsilon amino caproic acid (EACA) was given just after revascularization of the liver in Patient 2, but thrombogenic agents were not given to the others.

Methicillin or one of the other narrow spectrum antistaphylococcal drugs were started intravenously before or during operation and continued afterwards. In addition, agents were given which are usually effective against gram negative organisms; these included ampicillin, cephalothin, kanamycin, chloramphenicol, and polymyxin. The choice of antibiotics was made on the basis of prior cultures from the recipients' skin, trachea, urine, and feces. Additional cultures were taken during operation from the donor and recipient duodenum and from the donor gallbladder bile. In the event of growth from these locations, an adjustment in therapy based upon sensitivity data was made within 24 hours. In two cases, there was enough time to attempt preoperative sterilization of the recipient gastrointestinal tract with a 6 and 48-hour course, respectively, of oral Neomycin, Polymyxin, Cloxacillin and Mycostatin.

Postoperatively, cultures were taken at least twice a week from the wounds, nose, throat, urine, and feces in order to have a continuous record of the endogenous bacterial flora. In addition, blood cultures were obtained 3 or more times a week since it was soon learned that peripheral bacteremia could be the first sign of liver sepsis. Frequent changes in antibiotics were made according to the sensitivity of the bacteria in these various locations.

In addition, weekly or biweekly swabs from the nasopharynx, throat, rectum, and urine were obtained for virus isolation. The swabs were immersed in veal infusion broth containing 0.5% bovine serum albumin; 0.1 ml. was inoculated into monolayer cultures of human diploid fibroblasts (WI-38), primary rhesus monkey kidney cells, and H-Ep[2] cells. The cultures were observed daily for cytopathic effect and tested weekly for hemadsorption with guinea pig erythrocytes. One blind passage was performed for each specimen in each system.

The general plan of immunosuppressive therapy is shown in Figures 5 to 9. Azathioprine, prednisone and heterologous antilymphocyte globulin[24] were started during the operation. The maintenance doses of azathioprine were usually less than 1 mg./Kg. per day. In Cases 1-4 the quantities of prednisone were rapidly reduced during the first few postoperative weeks (Figs. 5-7) but in Cases 5-7 the steroid withdrawal was at a much lower rate (Figs. 8, 9). The ALG was given daily for the first one or 2 weeks and then every second or third day. ALG was stopped before the end of the planned 4-month course of therapy in Patients 2 and 4 because of the development of high titers of precipitating antibodies against equine horse protein.[12] Actinomycin C (40 or 60 μg.) was intermittently given to Patients 2-5 (Figs. 6, 8). The homografts of Patients 1-5 were irradiated with 2 or 3 doses of 100 or 150 R at depth on alternate days beginning 3 to 13 weeks post-transplantation (Figs. 5-8). The indication for either local x-ray therapy or Actinomycin was rejection.

The function of the transplanted livers was followed with a battery of

TABLE 1. *Data on 7 Recipients of Orthotopic Liver Homografts and Their Cadaveric Donors*

Case No.	Age	Sex	Weight (Kg.)	Blood Group	Disease	Time Death to Organ Revascularization	Terasaki Mismatches Major Groups	No. Sera
1. Donor	18 mth	M	6.4	O+	Microcephaly; aspiration pneumonitis	311 min	0	4/60
Recipient	19 mth	F	10.8	O+	Hepatoma			
2. Donor	48 mth	F	13.0	O+	Drowning	282 min	2 (Mac)	14/60
Recipient	20½ mth	F	8.7	O+	Biliary atresia*			
3. Donor	18 mth	F	8.2	A+	Acute CNS	271 min	5, 7	8/60
Recipient	13 mth	F	9.4	A+	Biliary atresia*			
4. Donor	20 mth	F	7.3	A+	Krabbe's disease	252 min	2 (Mac)	9/60
Recipient	14 mth	F	7.5	A+	Biliary atresia*			
5. Donor	14 mth	M	8.5	A+	Werdnig-Hoffman's disease	242 min	5, 7	23/60
Recipient	16 mth	F	7.5	A+	Biliary atresia*			
6. Donor	33 mth	M	11.4	O+	Meningitis	323 min	3	11/60
Recipient	24 mth	M	11.0	O+	Biliary atresia*			
7. Donor	27 yr	M	82.0	O−	GSW of brain	427 min	2 (Mac)	8/60
Recipient	16 yr	F	39.0	O+	Hepatoma			

* Extrahepatic.

standard hepatic function tests. In addition, the same clotting studies performed during operation were repeated at one to 4 week intervals. Liver scans using a technetium sulfide technic[6] were obtained every one to 3 weeks or more often if indicated.

Pathological Studies. Paraffin sections were routinely prepared from all tissues. In some instances additional material was fixed in Palade's buffered osmium tetroxide for subsequent electronmicroscopy and in liquid nitrogen for later examination by fluorescent antibody methods. The thin sections for electromicroscopy were stained with lead hydroxide and examined in a Phillips EM 300. Sections of the frozen tissue were tested by Drs. Andres, Hsu, and Seegal of Columbia University, with antisera prepared against human immunoglobulins G, M, and A, BIC/BIA globulins, C'1q and fibrinogen. Appropriate controls were also used.

Results

Survival. Patients 2, 3, 4 and 5 died 133, 186, 60, and 105 days post-transplantation. The three others are still alive with excellent liver function after 269, 68, and 33 days.

Two of the deaths (Cases 4 and 5) occurred within a few days after and were caused by the septic hepatic infarctions to be described below

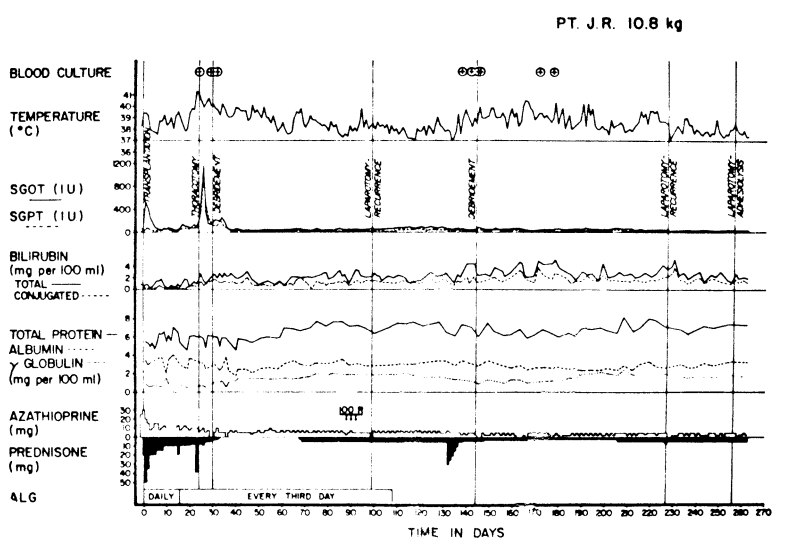

Fig. 5.— Course in Case 1. The patient is still alive 9 months after orthotopic liver transplantation. The indication for operation was hepatoma. Note the high rises in SGOT and SGPT, and the bacteremia which occurred at the time of the initial liver infarction. The later septicemia developed when the patient became leukopenic during treatment with vincristine for tumor metastases. The thoracotomy was for removal of an unexpanded right upper lung lobe. The first two laparotomies were for excision of tumor recurrences. Liver function has been stable for 8 months. 100 R—depth dose of local homograft irradiation. The temperatures are the highest for each day.

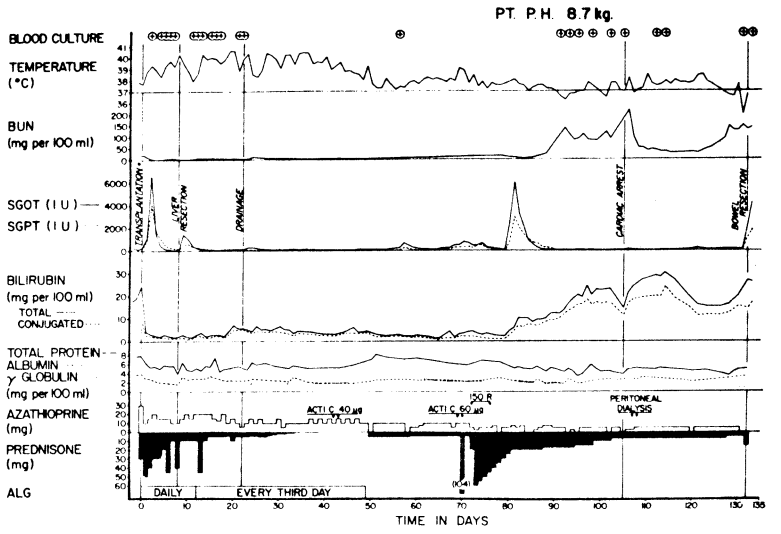

Fig. 6.— Case 2. The original disease was extrahepatic biliary atresia. A double arterial supply was connected as shown in Figure 4B. The right artery thrombosed within 2 days necessitating hepatic resection, and subsequent drainage of a subhepatic abscess. Eventually hepatic and renal failure developed, the latter leading to hyperkalemia and cardiac arrest. Bacteremia and later fungemia were intermittently demonstrated until eventual death from massive intestinal necrosis. Survival was 4-1/2 months. Acti C-Intravenous actinomycin C in micrograms. 150 R-depth dose of homograft irradiation. The temperatures are the maximums for each day.

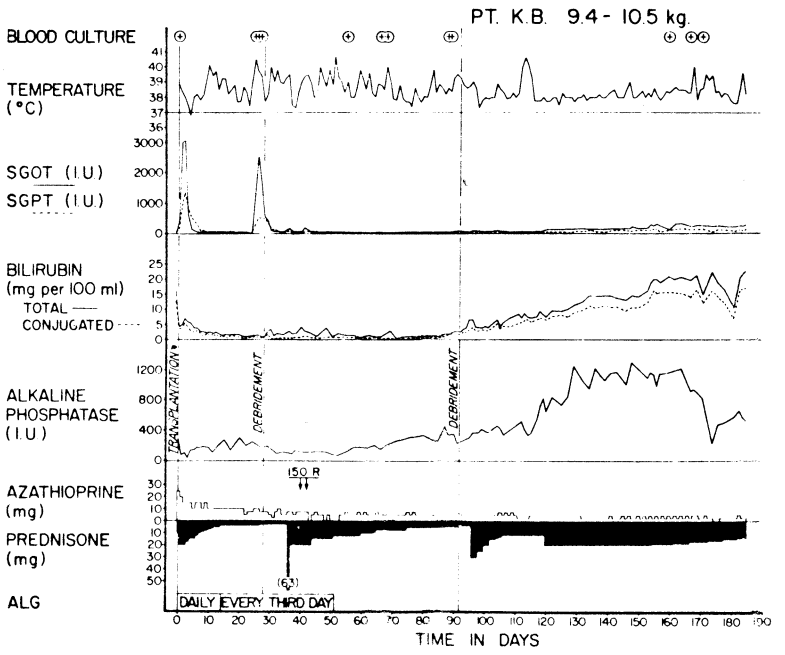

Fig. 7.— Course in Case 3 after orthotopic liver transplantation for extrahepatic biliary atresia. Hepatic function was excellent during the first 3 months despite infarctions of large portions of the central and right liver which were treated with debridement. Thereafter, liver failure was progressive. Note the late parallel increases in alkaline phosphatase and bilirubin. The immediate cause of death was intraperitoneal rupture of an undrained residual abscess. Survival was 6 months. Liver irradiation was with 150 R depth dose at each arrow. The temperatures are the daily maximums.

(Fig. 8). The delayed consequences of the same complication were largely responsible for the unfavorable outcome in Cases 2 and 3.

Technical Complications. The iatrogenic right diaphragmatic paralysis in Patients 1-3 persisted for 10, 7, and 2 weeks. In Case 1, failure of the right upper pulmonary lobe to expand prompted its removal during the fourth postoperative week (Fig. 5). The subsequent prevention of operative injury to the right phrenic nerve in Cases 4-7 was mentioned earlier.

In performing recipient hepatectomy, the small bowel in one case and the right transverse colon in another were perforated during mobilization of intestinal loops that had been caught at the site of previous hilar dissections. The rents, which were closed in 2 layers, healed without

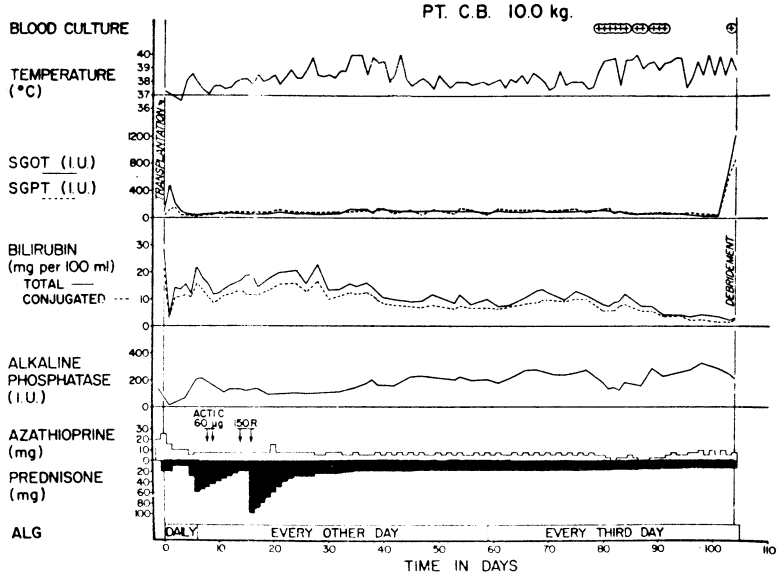

Fig. 8.— Course in Case 5 after orthotopic liver transplantation for extrahepatic biliary atresia. A vigorous and protracted rejection began within a few days postoperatively which was not reversed for 10 weeks. Function was improving when persistent gram negative septicemia presaged liver sepsis. Complete right lobar infarction finally occurred causing death within 48 hours. At autopsy the right hepatic artery was thrombosed. Acti C-intravenous actinomycin C in micrograms 150 R- homograft irradiation.

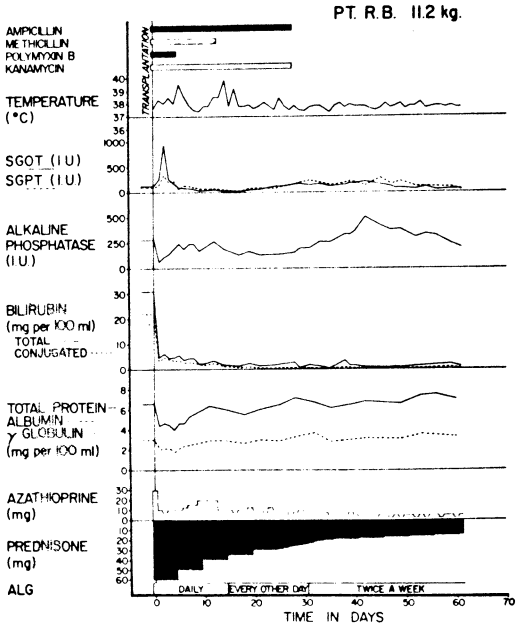

Fig. 9.— Course in Case 6. The operation was for extrahepatic biliary atresia. The homograft was immobilized by suturing its ligaments. Jaundice has not recurred, although rejection was diagnosed from the third to seventh postoperative weeks because of the rises in alkaline phosphatase, SGOT, and SGPT; these changes have receded. By 4 weeks, all antibiotic therapy was stopped. The child has since been afebrile. The temperatures are the maximums for each day. An equally benign course has been observed in Case 7.

incident.

Earlier attempts at liver transplantation were complicated by fibrinolysis, hypofibrinogenemia, thrombocytopenia and hemorrhage.[11,22,27] The same clotting abnormalities were seen in the more recent cases as well as depression of a number of discrete clotting factors produced within or outside the liver; these findings are being reported in detail elsewhere.[9] Nevertheless, bleeding was not a difficult problem. In the infants, the estimated blood loss ranged from 460 to 1,200 ml. (average 790 ml.). The 16-year-old girl received 4,000 ml. transfusion, almost all as the result of hemorrhage during hepatectomy.

Homograft Sepsis. Two to 104 days after transplantation, five of the seven patients developed gram negative septicemia with a variety of microorganisms including *Aerobacter-Klebsiella, Escherichia coli, and Bacteroides*. Bilirubin, alkaline phosphatases, serum proteins, and prothrombin times were little affected if at all, but there were high rises in the SGOT and SGPT (Figs. 5-8). Liver scans showed large defects, involving principally the right lobe (Figs. 10, 11). Each of the patients had developed a septic hepatic infarct.

This complication was not easily controlled in spite of specific antibiotic therapy and excisions, extraperitoneal debridements (Fig. 12). and drainage. Two of the five patients died within a few days. The other three, who initially survived the complication of regional hepatic gangrene, had a protracted subsequent morbidity. The tracts running to the subphrenic space (Case 2) or within the liver (Cases 1 and 3) were irrigated and probed every day. They could not be kept sterile in spite of local and systemic antibiotics. The same organisms cultured from the draining wounds appeared from time to time in the blood stream necessitating reoperation and more extensive debridements (Figs. 5-7). At these times it was noticed that plugs of dead tissue radiated from the main tracts, often surrounding thrombosed intrahepatic portal triads. Between debridements there was variable evidence from the scans that filling in of the defects was occurring as the consequence of hepatic regeneration (Figs. 10, 11). Nevertheless, the open wounds never completely healed, even after more than 8 months in Case 1.

Early Liver Function. During the first few postoperative days, all seven of the patients had moderate but rapidly reversible rises in SGOT and SGPT. In spite of this evidence of ischemic injury, the livers provided life sustaining function from the beginning. The two recipients with normal bilirubin values before operation did not become jaundiced within the first

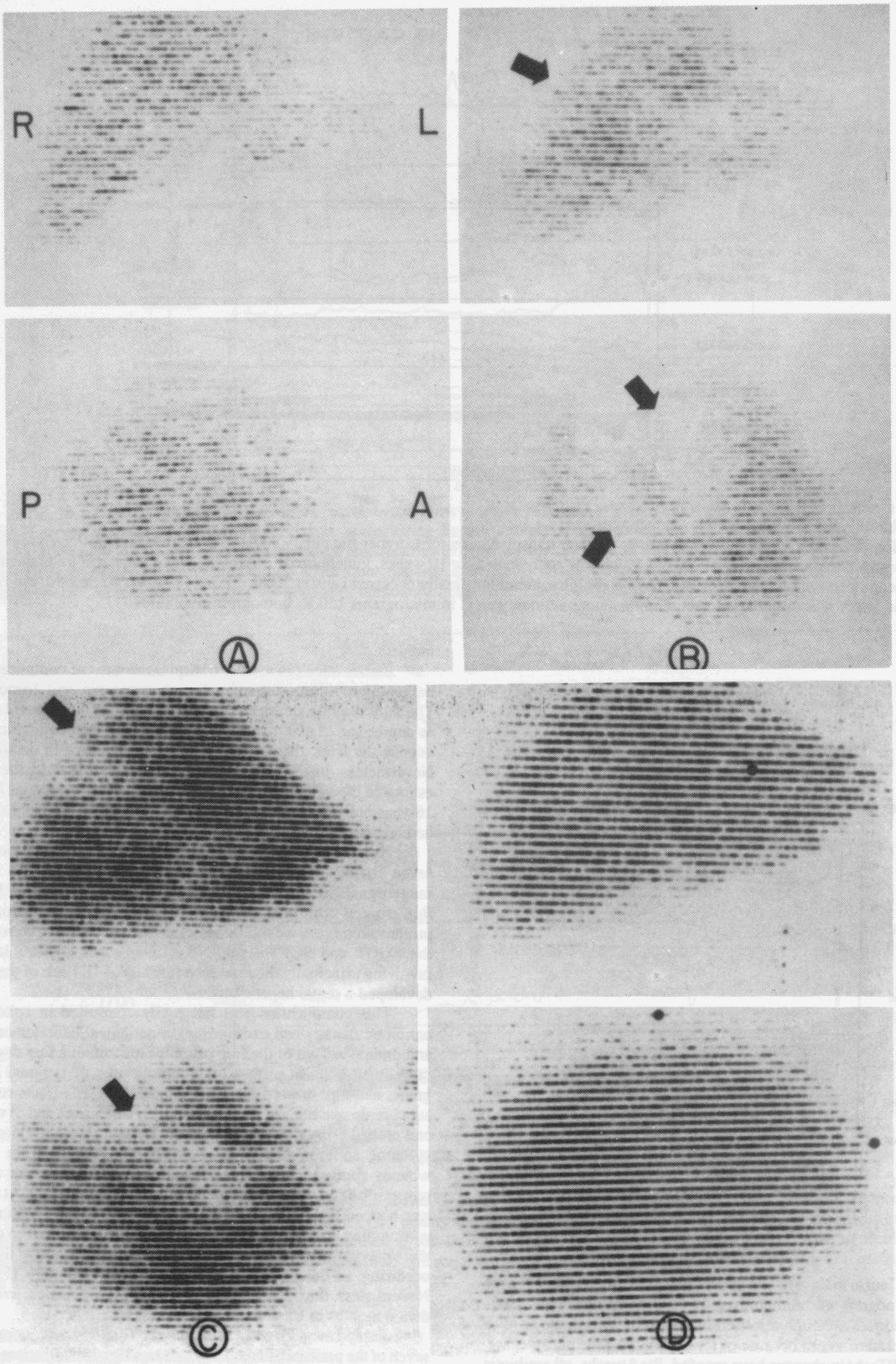

Fig. 10.— Radioisotope liver scans in Case 1 obtained with 99M-technetium. A — Seventeen days after transplantation. The isotope uptake is diminished but there are no defects. B — Large non-opacifying areas are demonstrable (arrows) 29 days after transplantation in the posterior part of the right lobe. The patient had septicemia. C— Three days later. The necrotic liver tissue had been debrided. D — The defects are no longer seen 252 days after operation. Note that the liver has progressively increased in size.

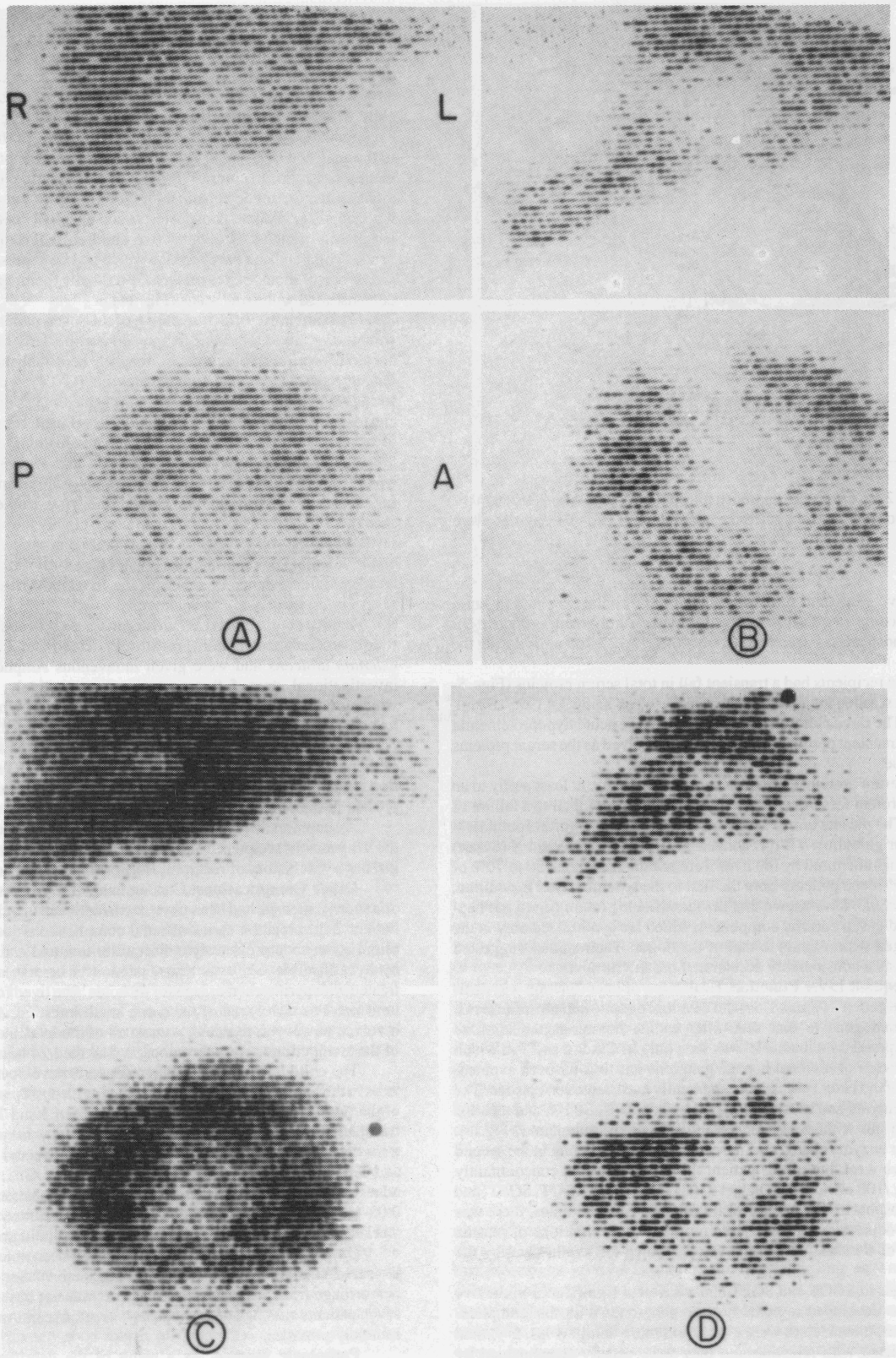

Fig. 11.— Radioisotope liver scans in Case 3. A — Normal scan 10 days post-transplantation. B — Non-opacifying areas in the right and central parts of the liver 27 days postoperatively. Septicemia had developed. C — The necrotic areas were debrided. There was evidence of regeneration 84 days post-transplantation. Liver function was still excellent. D — Liver shrinkage and diminished isotope uptake 176 days after transplantation. The child died 10 days later when an abscess in the left lobe ruptured into the peritoneal cavity.

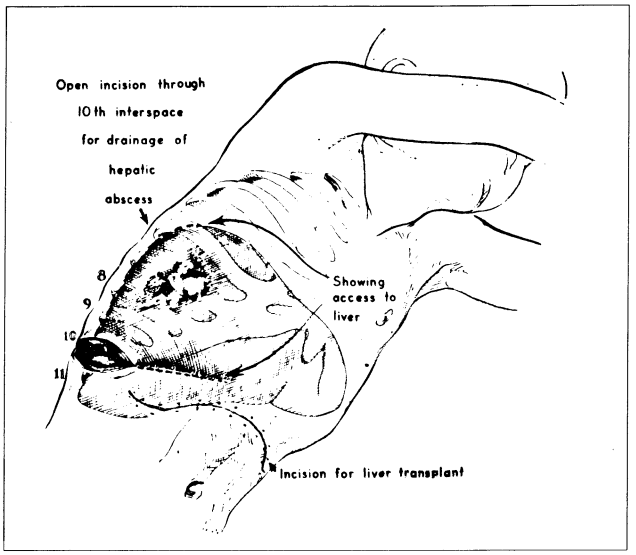

Fig. 12. — Operative approach to septic liver infarctions through the 10th intercostal space. Neither the pleural or peritoneal cavities were entered.

postoperative week and the five patients with biliary atresia had rapid partial or complete disappearance of hyperbilirubinemia (Figs. 6-9). Alkaline phosphatases decreased from 140-632 to 27-165 units during the first week.

All the recipients had a transient fall in total serum proteins (Figs. 5-9), the lowest value in the first postoperative week being 3.4 Gm./100 ml. in Case 3. The three children with the most severe acute hypoproteinemia developed transient peripheral edema which resolved as the serum proteins began to rise.

The serum protein depression appeared to be due at least partly to an increased protein loss or heightened catabolism, rather than to a failure of synthesis. The plasma levels of Factors I (fibrinogen), II (prothrombin), V (Accelerator globulin), VII (proconvertin), IX (Christmas) and X (Stuart) which are manufactured by the liver were maintained above 50 to 70% of normal in all seven patients from the first to the seventh days.[9] In addition, Kashiwagi *et al.*[13] have shown that the identifiable protein genotypes haptoglobin and group specific component, which are synthesized only in the liver, changed within hours to that of the donor. Their studies suggested that there was a considerably accelerated protein turnover.

Subsequent Liver Function. Evaluation of later function was difficult in all the patients (Cases 1-5) who developed septic hepatic infarctions. A course analogous to that seen after canine homotransplantation to immunosuppressed recipients[21,25] was seen only in Cases 6 and 7 in which the complication of regional hepatic gangrene has thus far been avoided.

Both of the latter two patients apparently had a rejection episode. The first had moderate and reversible increases in SGOT, SGPT, and alkaline phosphatase but without the recurrence of hyperbilirubinemia (Fig. 9). Very similar enzyme changes were seen in Case 7 beginning in the second postoperative week but in this patient the serum bilirubin concomitantly rose to 6 mg./100 ml. and then began to fall again as the SGOT, SGPT, and alkaline phosphatases returned toward normal. At these times, there was little or no depression in either patient in the concentrations of plasma proteins or of 6 measured clotting factors which are synthesized by the liver.

Changes in SGOT and SGPT had a different significance in the five patients who developed regional hepatic gangrene. With this complication, the transaminase rises were higher and more abrupt (Figs. 5-8) than those associated with rejection and they were usually accompanied by minimal acute alterations in other measures of liver function. In Cases 1-3 there was little other evidence to support the diagnosis of rejection at that time (Figs. 5-7). Difficult rejections had already occurred in Cases 4 and 5 but these appeared to have long since been reversed at least in terms of resolution of jaundice (Fig. 8).

In the three patients who did not die as the immediate consequence of septic liver infarction, it was difficult to differentiate the subsequent adverse effects of this complication from those of chronic rejection. The child whose preoperative diagnosis was hepatoma (Fig. 5) has had stable excretory and synthetic hepatic function (including normal levels of all six of the analyzed liver-based clotting factors) in the 8 months since debridement procedures. In contrast, progressive liver failure developed about 2 months after the septic hepatic infarctions in Cases 2 and 3 (Figs. 6 and 7), with jaundice, alkaline phosphatemia and eventual depression of all studied plasma proteins. The latter two children redeveloped the extensive venous collaterals on the abdomen which had been present preoperatively and in one of them ascites recurred; eventually, homograft shrinkage was demonstrated with liver scans (Fig. 11).

Extrahepatic Infections. Five of the seven patients had evanescent parahilar or lower lobar infiltrations during the early course, for which a bacterial or viral etiology could not then be established. Subsequently, Patient 5 persistently had cytomegalovirus (CMV) in tracheal excretions from postoperative days 75 to 105 at the same time as diffuse pneumonitis and *Aerobacter-Klebsiella* bacteremia developed (Fig. 8). At autopsy, there was extensive bilateral pneumonia and histologic evidence of lung infestation with bacteria, cytomegalovirus, and *Pneumocystis carinii*. In another patient (Case 3) chronic pneumonitis was evident radiographically for the last 3 months of life. Fungal and viral studies of the autopsy specimens have thus far been inconclusive. Finally the second infant in the series had CMV in tracheal secretions on two occasions but never developed symptoms or abnormalities of her chest x-ray. At autopsy, there was no histologic evidence of CMV in the lungs but there was moderately severe infestation with *Pneumocystis carinii*.

Transient fungemia was detected in Cases 1, 2, and 4 during the sixth, fourth, and first postoperative months. Patients 1 and 2 were infected with *Candida albicans* and were given intravenous Amphotericin B and an investigational drug 5-fluorocytosine,* respectively; the latter agent which is less nephrotoxic than Amphotericin was used because the patient had azotemia. Patient 4, who had an unidentified yeast, was treated with Amphotericin B. In all three cases the fungemia ceased after the beginning of therapy. Two of the children subsequently died. In one, a fungal infection could not be found at autopsy. Multiple fungal brain abscesses were present in the other.

Asymptomatic *Herpes simplex* virus excretion was demonstrated in the 16-year-old recipient, beginning 3 weeks after transplantation. This girl has a past history of recurrent *Herpes simplex* mucocutaneous lesions.

Other Complications. Concomitant with progressive liver failure, and shortly after she had been treated wtih both kanamycin and Polymyxin, Patient 2 developed a series of renal complications including azotemia, oliguria, secondary electrolyte disequilibrium, and cardiac arrest caused by hyperkalemia. She was resuscitated with cardiac massage and then treated with peritoneal massage for 48 hours. She lived for another 4 weeks until necrosis developed of the entire small intestine. At exploration the involved bowel was resected. Almost all of the intestinal veins and many of the small arteries were thrombosed. She died 24 hours later.

The child treated for hepatoma (Case 1) developed multiple metastases in the lungs and abdomen during the fourth postoperative month. Two of the intra-abdominal recurrences were excised 3 and 7-1/2 months after transplantation. The first which weighed 28 Gm. was superior to the right transverse colon. The second, which partially obstructed the sigmoid colon and left ureter at the pelvic brim, weighed 164 Gm.; one month later adhesiolysis was necessary because of intestinal obstruction. A course of 0.02 to 0.1 mg./Kg. of intravenous vincristin sulfate (Oncovin®) for 7 weeks, started in the fourth postoperative month, did not halt the growth.

Gastrointestinal bleeding has previously been noted after orthotopic liver transplantation in dogs,[9,21,25] pigs,[17] and man.[19,23] Serious postoperative hemorrhage from the gastrointestinal tract was not observed in any of the seven patients although occult melena was often seen. Antacid therapy was routinely provided.

Pathologic Studies. As a result of the debridement procedures, homograft tissue became available from 8 to 54 days after transplantation

* Roche Company, Professional Service Department, 212 Oxford, New York, N.Y.

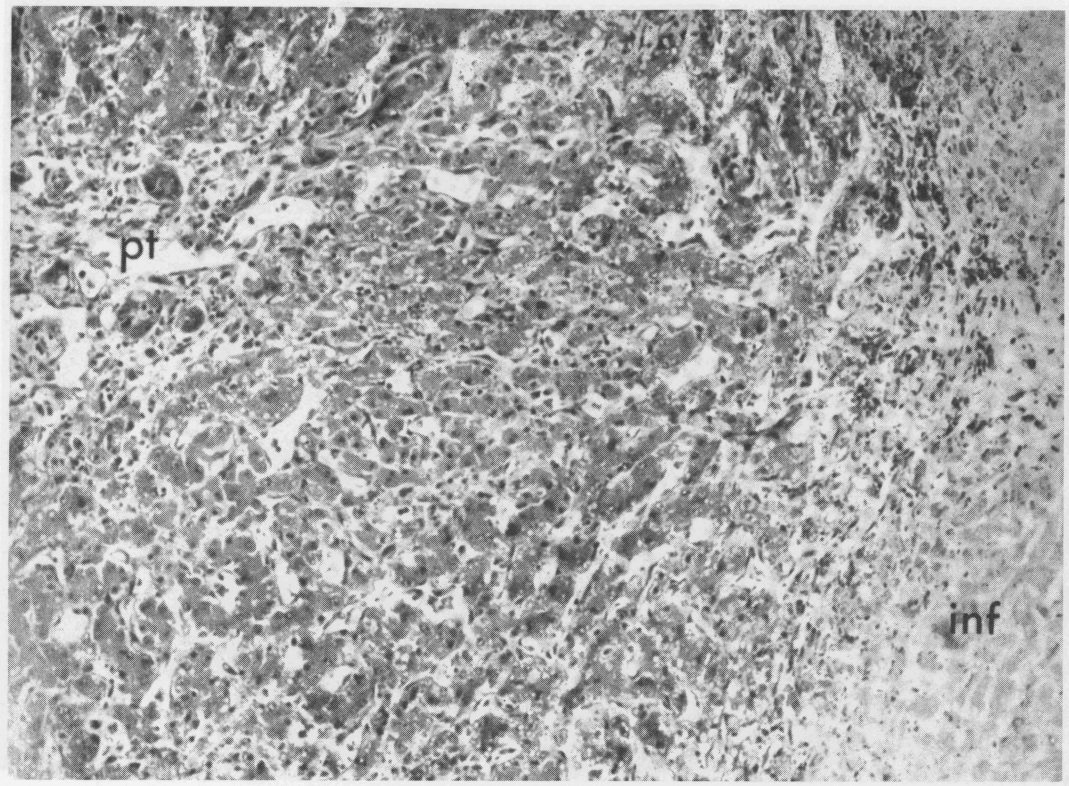

Fig. 13.— Hepatic homograft from Patient 4 two months after transplantation. A large infarct (inf) in the right lobe is separated from the viable liver by a rim of fibroblasts and small lymphocytes. There is excess fibrous tissue and several small bile ductules in the portal tract (pt). H & E (X 180.)

in Cases 1-4. A biopsy was obtained after 100 days from the liver of Patient 1. The homografts of Patient 2, 3, 4, and 5 were removed at autopsy.

The pieces of liver removed from Patients 1-5 at debridement of the right hepatic lobe all contained multiple infarcts. In the necrotic centers of many of these there were large numbers of gram negative bacilli. The infarcts were rimmed by abundant neutrophile polymorphonuclear leukocytes, many eosinophiles and some lymphoid cells. Multiple infarcts were also present in the hepatic homografts removed from Patients 2-5 at autopsy. The areas of dead tissue were predominantly in the right lobe and were surrounded by a zone of proliferating fibroblasts and by small lymphocytes (Fig. 13). Several of the infarcts contained gram negative bacteria. In Patients 2, 3, and 4 thrombosis of the right hepatic artery could be seen with the naked eye; in Patient 5 microscopy showed obliteration of this part of the arterial tree by organized thrombus.

The pathological changes in the noninfarcted parts of the liver varied in degree in the five cases. The biopsy obtained from Patient 1, 100 days after hepatic transplantation, was the least abnormal (Fig. 14). In the portal tracts there was a moderately dense focal cellular infiltration. The majority of these cells were small lymphocytes, but about 5% were plasma cells with IgM in their cytoplasm. Several were neutrophile polymorphs; a few were eosinophiles. There was a slight increase in the amount of portal reticulin and collagen and new bile ductules were present in the portal tracts. The Kupffer cells were prominent but not increased in number. A few of the centrilobular bile canaliculi contained small bile "thrombi." There were no alterations in the fine structure of the hepatocytes and the blood vessels were normal. The lack of important pathological changes in this liver correlated well with the good match of donor and recipient shown by lymphocyte typing.

Similar, but slightly more pronounced changes, were present in the liver homografts removed from Patients 4 and 5 at 2 and 3-1/2 months, respectively, after transplantation (Fig. 15). Although the portal fibrosis was marked there was no linking of adjacent tracts. There were many proliferating small bile ductules and in Patient 5 the bile ducts were dilated and filled with casts that were composed of inspissated bile, surrounded by neutrophile polymorphs. Cellular infiltration in these livers was slight.

The most severely damaged homografts were those from the long surviving Patients 2 and 3. In Patient 2 the portal fibrosis had progressed to cirrhosis. There was linking of portal tract to portal tract and portal tract to central vein by bands of fibrous tissue. The normal lobular architecture of the liver had been lost and there were small regeneration nodules. Areas of liver cell necrosis were present (Fig. 16). Some hepatic artery branches showed slight to moderate degrees of fibrous intimal thickening. In Patient 3 there was portal fibrosis but not cirrhosis. Many of the small branches of the hepatic artery were greatly narrowed or obliterated by intimal thickening. The thickened intima contained collagen and large fat-laden cells. In some of the affected vessels the internal elastic lamina was ruptured. Cellular infiltration was slight, there being only a few foci of small lymphocytes in the portal tracts. This was in contrast to the dense lymphoid cell infiltration seen in the hepatic tissue removed from this same patient 27 days after transplantation.

Discussion

Survival in all 7 of the presently reported cases exceeded that obtained previouly in human trials.[5,16,19,22,23] The results have established that the actual operative procedure can be done in man with relative safety and that subsequent survival is possible for at least as long as 9 months. The course after transplantation of these children was markedly different than that of earlier recipients of orthotopic liver homografts inasmuch as all but one of the patients had a very satisfactory early postoperative convalescence. As discussed elsewhere,[20] the difference was probably due principally to several improvements in care including more discriminating donor selection, the employment of an efficient method of interim organ preservation, and improved immunosuppressive agents.

Three of the seven patients are still alive 9 months, 2-1/3 months, and

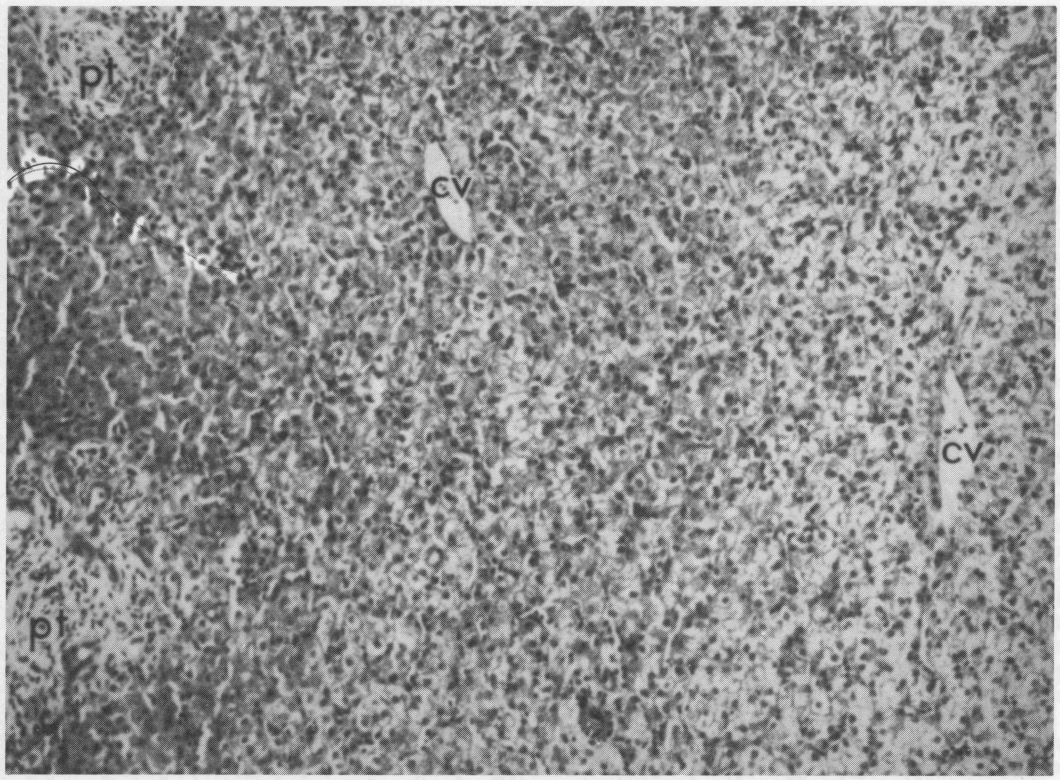

Fig. 14.— Patient 1. Biopsy of hepatic homograft 100 days after transplantation. In the portal tracts (pt) the amount of fibrous issue is increased and there are small bile ductules and a few infiltrating lymphocytes. The central veins (cv) and hepatocytes appear normal. H & E (X 180.)

one month after transplantation. The other 4 died after 2, 3-1/2, 4-1/2, and 6 months. In each case of the unsuccessfully treated recipients, the development of a septic liver infarction either caused or significantly contributed to the unfavorable outcome. Even the child who is alive after 9 months developed the same life threatening complication. As reported earlier,[20] the sequence of events seemed to be, first, infarction of portions of the homografts and, then, overgrowth of gram negative bacteria in the necrotic areas. Fortunately, progress has been made in understanding the etiology of these disasters.

Clearly an important factor is the natural susceptibility of the liver to bacterial contamination via the portal vein or biliary tract. In a recent study in dogs and pigs which were not given antibiotic therapy[3] it was shown that the incidence of positive cultures from liver tissue was increased after simple sham operations in which the liver was not disturbed. When an ischemic injury was added by performance of simulated autotransplantation, all livers became contaminated. The bacteriologic changes were even more pronounced in liver homografts transplanted either to unmodified or immunosuppressed recipients. It was concluded that the number of bacteria in the liver reflected more or less accurately the magnitude of hepatic injury from mechanical causes as well as from rejection. These studies help explain the previously reported variable incidence of liver abscess formation after autotransplantation[1] or homotransplantation of the canine liver[21,25]; presumably, any necrotic focus could become infected and serve as the beginning of an abscess cavity (Fig. 17) particularly if the host were being given immunosuppressive therapy. They do not, however, fully explain the syndrome encountered in five of the seven human recipients.

The patients did not have discrete abscesses. Instead, the precipitating event seemed to be dearterialization of part or all of the right hepatic lobe. Studies of the debrided necrotic tissue[20] suggested acute ischemic infarctions in the right lobe which in some areas had not yet become invaded by microorganisms. Furthermore, thrombosis of the right hepatic artery was proved either at operation or autopsy in all four of the patients who died. In another child recently treated by Fonkalsrud,[7] the same kind of selective thrombosis of the right hepatic artery was the cause of death 2 weeks after liver replacement for extrahepatic biliary atresia.

In an earlier report,[20] we speculated that the ischemic infarcts might be a relatively isolated manifestation of uncontrolled acute homograft rejection. It was suggested that the hepatic blood flow, which has been shown to decline sharply in dogs at this time,[10] might have dropped in some regions below a life sustaining level. A similar hypothesis, based on angiographic studies in dogs, had previoulsy been advanced by Moore *et al.*[16] As a primary explanation, this proposition has since lost much of its persuasiveness in view of the aforementioned observations from the four autopsy examinations.

In these four homografts, including the two which were examined 2 and 8 days after thrombosis of the right lobar arterial supply, there was little or no evidence of active rejection in the uninfarcted portions of the liver. The concept that immunologically mediated reductions in blood flow should specifically effect the right portion of the organ is further weakened by the fact that this kind of selective regional vulnerability of the liver has never been reported in animal studies and probably does not occur, at least in dogs and pigs. In the canine and porcine bacteriologic experiments cited above[3] and in a separate investigation of liver scanning in dogs,[8] lobar and segmental necrosis were specifically looked for. There was not a single example in 32 experiments.

There is now evidence from studies in fresh cadavers that mechanical factors predispose man (or at least small children) to a right lobar vascular accident, possibly because of the erect position used by humans. In humans the right hepatic artery is longer than the left branch (Fig. 18A) and usually traverses behind the other central hilar structures where it is held by surrounding tissues. When the restraining ligaments of the liver are cut and when the vascular structures entering and leaving the liver are skeletonized, the right lobe rotates on the fulcrum of the vertebral column and recedes to a somewhat more posterior and inferior location than normal. If the head of the x-ray table is elevated to 60°, a sharp kink of the proximal right hepatic artery can be demonstrated (Fig. 18B). Presumably such a

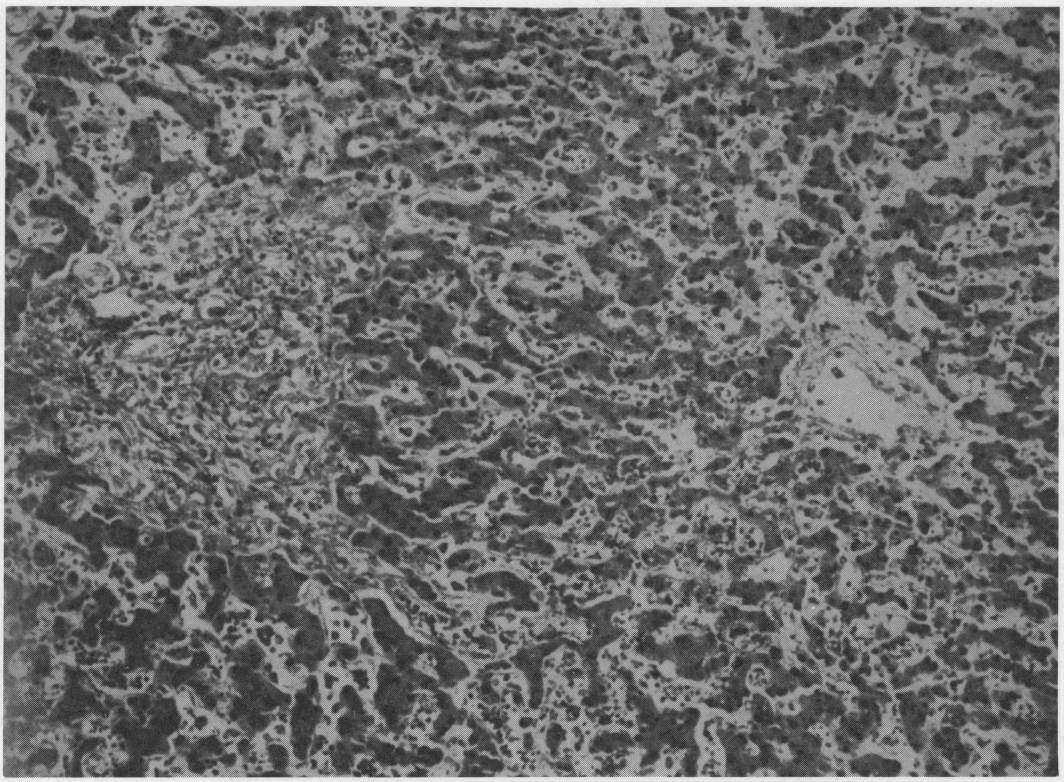

Fig. 15.— Patient 5. Hepatic homograft removed at autopsy 3-1/2 months after transplantation. The portal fibrosis is more marked than in Patient 1. The Kupffer cells are prominent. (H & E X 180.)

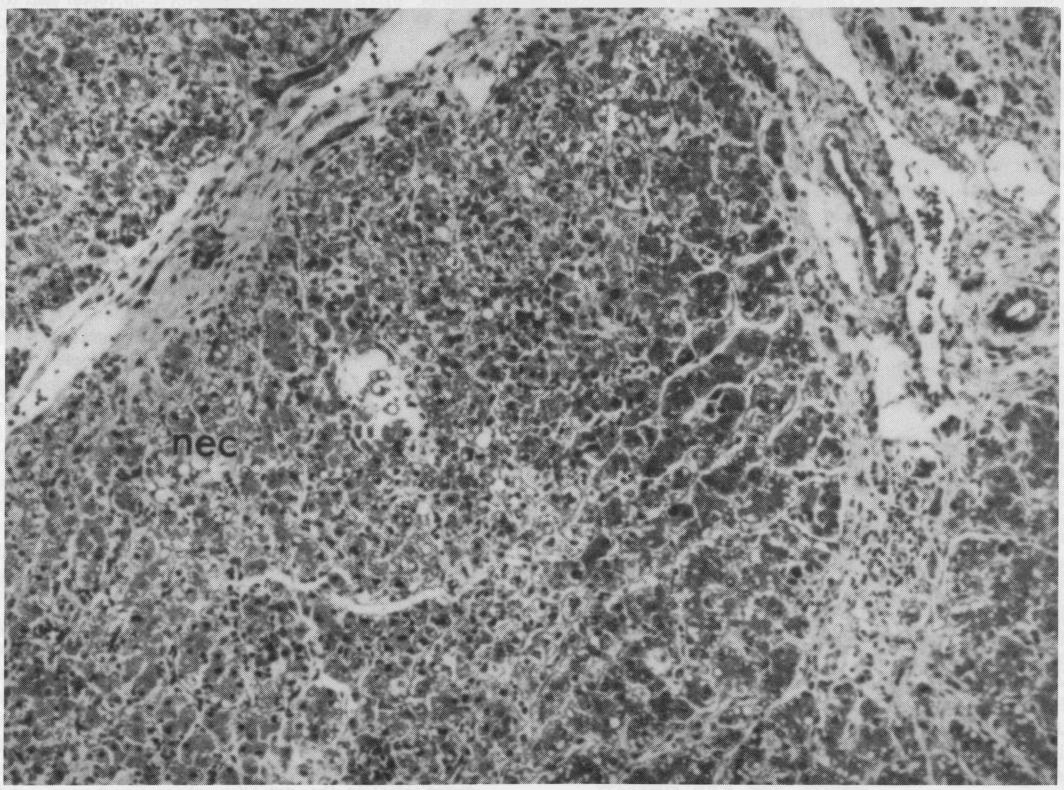

Fig. 16.— Patient 2. Liver graft removed at autopsy 4-1/2 months after transplantation. The normal lobular architecture is interrupted by bands of connective tissue. Part of the liver is necrotic (nec.) (H & E X 180.)

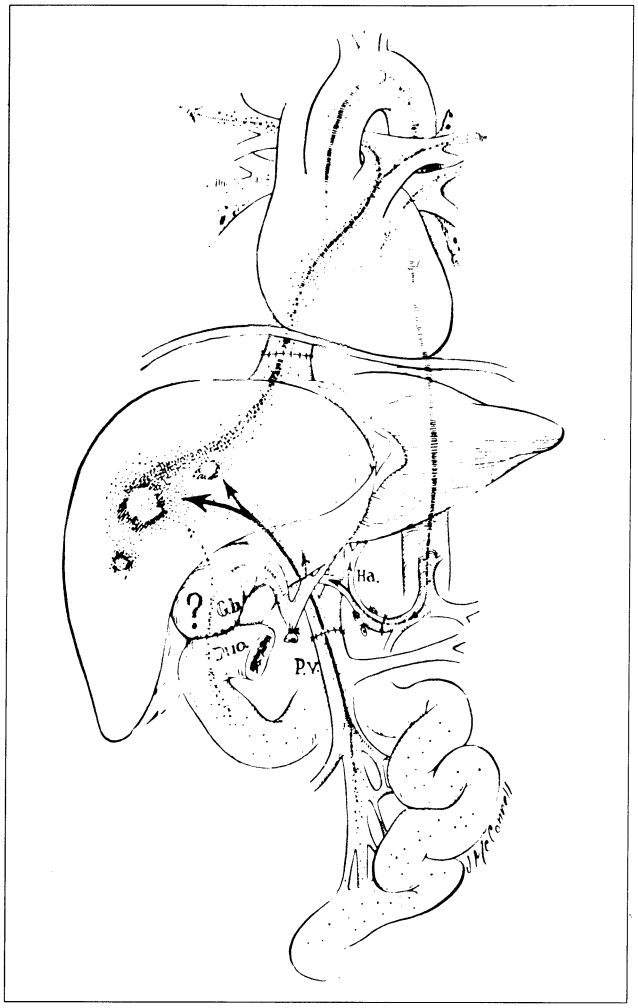

Fig. 17.— An anatomical explanation of the predisposition of the liver to bacterial sepsis. Presumably the invading microorganisms enter via the portal vein or through the reconstructed biliary tract.

dangerous situation could be further aggravated in the post-transplantation period either by the diminutions in blood flow or by the organ swelling which occurs with rejection. The constellation of circumstances might be especially hazardous in infant livers with their fine caliber thin-walled arterial branches.

Because of the possible contributory role of such mechanical factors in the production of right lobar infarction, the homograft was fixed in the desired position in the last two cases by firmly reattaching its falciform ligament to the same ligament in the recipient. Thus far, these two patients have not had any evidence of acute liver necrosis during the follow up studies of one and 2-1/3 months, respectively.

Compared to the septic liver infarctions, the other complications seen in the patients seemed relatively trivial. The mild intraoperative bleeding diathesis required no therapy. The acute acidosis which occurred at about the same time[20] was easily managed. The right hemidiaphragmatic paralysis which was probably caused by crushing the right phrenic nerve in Patients 1-3, was transient (10 to 50 days); furthermore it was prevented in the next four cases.

Certain other complications which plagued earlier attempts at orthotopic liver transplantation were eliminated in the present series. Postoperative gastrointestinal hemorrhage did not develop in any of the patients. Pulmonary embolization, which was either the primary or an important contributory cause of death in three of the first five recipients of orthotopic liver homografts,[22,23] did not occur at all. In the earlier cases, the clots originated at or near the lower venotomy sites used for insertion of plastic bypasses.[23,27] At that time, such prostheses were thought necessary during the anhepatic phase for decompression of the blocked portal and vena caval systems. It is tragic to realize from the experience in the presently reported series that such external bypasses are not necessary.

Most importantly, the toxicity from immunosuppressive agents so often seen in our earlier experience[19,22,23] was avoided. There were no examples of bone marrow depression, probably because the azathioprine doses were generally kept at less than one mg./Kg. per day. Prednisone was initially given in large quantities but reduced thereafter as rapidly as possible. Heterologous antilymphocyte globulin (ALG) which in many dogs can delay or prevent liver homograft rejection when used as the only therapy,[24] was employed as an adjuvant agent in the same general way as has been reported after clinical renal homotransplantation.[24]

Patients 6 and 7 in this series have had only minor and easily reversible signs of rejection during their one and 2-1/2 month periods of follow-up study. Evaluation of the effectiveness of the foregoing immunosuppressive regimen was complicated in the other five cases by the development of extensive liver necrosis which, as discussed earlier, was not necessarily caused by rejection but which could be expected to adversely effect both hepatic function and structure. In one of the latter three patients who survived this complication at 23 days (Case 1), a liver biopsy 77 days later had only minimal abnormalities. The donor-recipient histocompatibility match in this case was the best one in the entire series; good liver function has been maintained during the entire 9 months after transplantation. The other two children who were saved by liver debridement or excision, only to die of hepatic failure 4 and 5-1/2 months later, had shrunken homografts at autopsy. Histologically, these organs had the kind of fibrosis seen in many long survivors after comparable canine liver transplantation.[21] However, there were no mononuclear cells or other stigmata of active rejection in the specimens.

Similarly, the principal abnormalities in the homografts from the two children who died after 2 and 3-1/2 months, within a few days after right hepatic artery thrombosis, were those of repair and to a lesser degree, regeneration. In each, the extent of fibrosis was moderate. Both of these patients had passed through difficult early rejections but had good liver function prior to the vascular accidents in the transplants; serum bilirubins were 2 and 2.3 mg./100 ml., respectively, just before death.

Reasonable conclusions from these combined clinical and pathological observations would seem to be that the immunosuppressive regimen used did not uniformly prevent the onset of rejection; that it permitted eventual control of the acute phase of this process; and that the degree of ultimate organ damage was a reflection of the severity of earlier rejection injury and in turn of the extent of donor-recipient histocompatibility. Efforts to improve the histocompatibility matches in future trials will depend upon characterization of the white cell antigen profile of a large number of prospective recipients from whom the appropriate choice could be made. In order to have a large enough recipient pool to permit fine selectivity, an inter-city network of collaborating physicians will be necessary such as that which is now being developed through our institutions.

Summary

Seven patients aged 13 months to 16 years were treated with orthotopic homotransplantation of cadaveric livers between July, 1967 and March, 1968. The indication for operation was congenital extrahepatic biliary atresia in five cases and carcinoma of the liver in the other two. The histocompatibility match between the donors and recipients ranged from poor to excellent. After donor death, the homografts received interim preservation with a technic that included hypothermia, hyperbaric oxygenation and perfusion with diluted blood. Postoperative immunosuppressive therapy was provided with azathioprine, prednisone, and heterologous antilymphocyte globulin (ALG). Four of the recipients died after 60, 105, 133, and 186 days. The other three are still alive after 9, 2-1/3, and one months. The improved results in the present series compared with those obtained in earlier human trials, seem to be a consequence of improved donor selection, the application of an effective organ preservation system and the use of an improved immunosuppressive regimen.

All seven of the recipients had good early liver function. In five of the

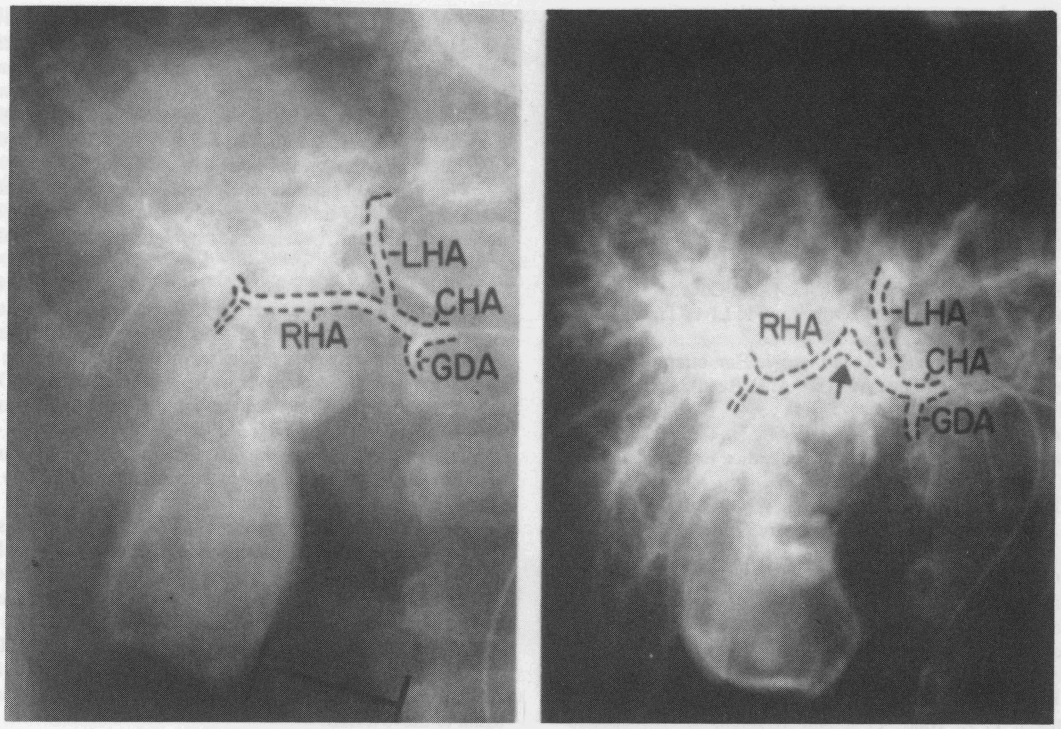

Fig. 18.— Angiographic studies performed in a 5-year-old child immediately after her death from head injuries. Dye was injected into the common hepatic artery (CHA) proximal to the gastroduodenal artery (GDA). Left — Initial injection. Note the smooth course of the right hepatic artery (RHA). Right — The restraining ligaments of the liver have been incised, a cholecystoduodenostomy performed, and the head of the x-ray table elevated to 60°. The right lobe of the liver has rotated down and medially. The course of the left hepatic artery is undisturbed. However, the right hepatic artery (RHA) is now severely kinked where it passes beneath the common duct (see discussion).

seven cases all or part of the right liver lobe underwent necrosis from 2 to 104 days after operation. Thrombosis of the right hepatic artery was proved in four of these homografts either at operation or autopsy; additional angiographic studies in cadavers suggested that the regional de-arterialization had an at least partially mechanical etiology. With invasion of the necrotic liver tissue by gram negative bacteria, the patients became profoundly toxic. Two of the recipients died within a few days, and two others died of progressive liver failure 4 and 5-1/2 months later.

In the 5 livers studied pathologically from 60 days to more than 6 months after transplantation, there was portal fibrosis, a slight infiltration by small lymphocytes around the portal vein branches, proliferation of small bile ductules and some central cholestasis. One of the two longer surviving homografts had progressed to cirrhosis and in both many of the small hepatic artery branches were narrowed by intimal thickening. These various changes are thought to be the result of rejections; they were least severe in the homograft which was shown by lymphocyte typing to be most compatible with its recipient.

Addendum

July 13, 1968 — The 3 patients surviving at the time the manuscript was submitted are still alive. The first patient will be one year post-transplantation in 10 days. The other 2 have now been followed for 5 and 4 postoperative months respectively. Three other recipients treated 3 months, 6 weeks, and 3-1/2 weeks ago are well. There have been no septic hepatic infarctions in the last 5 cases.

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Discussion

Dr. Francis D. Moore (Boston): Dr. Starzl's work in this field is absolutely outstanding and for those of us interested in liver transplantation, this is a banner day when we can contemplate four patients alive and well with lethal liver disease removed and a new liver in place. This is a magnificent achievement and liver surgery as of this day has an entirely new look from hither forward. But I would like to use this opportunity to discuss another aspect of Dr. Starzl's work.

Four years ago, at the Society of University Surgeons, when Dr. Starzl's initial clinical experience and ours likewise had ended with fatality, it was clear (and so stated at the meeting) that more progress was needed before it would be ethically, morally, surgically and scientifically acceptable to move ahead again with clinical liver transplantation.

Immunosuppression without hepatotoxicity was essential, as well as improved perfusion-storage. Now these things have come to pass, especially by the development of anti-lymphocyte globulin, and it is appropriate again to move ahead. If this forward motion is undertaken by persons or departments that have practical capability in transplant immunology and histocompatibility matching, as well as surgical experience with transplantation and the postoperative care of patients on immunosuppression, there is no need for consultant boards to declare the patient operable or a team from another institution to decide that the donor is dead.

The time-temperature curves for liver donation, the permissible normothermic dead-time, and the prior state of death are the same for liver donation as for heart, with the heart somewhat less vulnerable to normothermic ischemia.

There has recently been a statement issued by a committee in Washington that attempts to deal with these matters as applied to the heart. As nearly as I can see, none of the authors of this document has busied himself with the transplant problem over these last 15 years; the document makes no reference whatsoever to the long work of many Departments in this country who have labored through the difficult years of transplantation, nor does it acknowledge the obvious fact that actual experience in immunogenetics, immunosuppression and surgical transplantation are the essential normal prerequisites to moving ahead with any newly transplantable organ.

Nor does it make reference to the fact that the moral security of the next 25 years of American surgery, in exploring this new field, will rest secure just where it has in the past 20 years: in giving free and untrammelled opportunity for development to those Departments and individuals that are willing to take the time and trouble to develop both the immunological and surgical aspects of organ transplantation, as Dr. Starzl has demonstrated today with a project that was held in abeyance until the fundamentally ethical nature of science itself indicated that it was time again to move ahead.

Dr. Eric W. Fonkalsrud (Los Angeles): I would like to congratulate Dr. Starzl and his associates for their very excellent contributions to furthering the knowledge of both liver preservation and transplantation, and for their remarkably good clinical results, shown in this study.

We have followed Dr. Starzl's work with very great interest, however have used a slightly different technic in the laboratory and in one patient.

The emphasis in our studies has been on the use of hepatic cell stabilizing drugs, such as chlorpromazine and cortisone administered to the donor after death followed by external hypothermia to the graft after excision. A siliconized internal vascular shunt has been used to decompress the inferior vena cava and portal vein to the right atrium in the recipient.

This slide [slide] shows the liver of a 2-1/2-year-old boy with biliary atresis who underwent orthotopic liver transplantation. The total time of liver ischemia was 91 minutes.

This photograph shows how pink and uncongested the graft appeared 30 minutes after revascularization. The falciform ligament and suspensory ligaments of the grate were attached to the diaphragm in this patient, not so much to prevent arterial kinking as to provide support to the vena caval anastomosis.

The patient recovered from the operation rapidly and experienced a drop in bilirubin from 33 to 4.5 mg./100 ml. and alkaline phosphatase from 115 to 15 KA units within 24 hours. There was prompt good bile excretion which was drained externally. Immunosuppression was started on the day of operation and continued as in Dr. Starzl's study.

The patient was ambulatory and taking a regular diet when, on the fourteenth day, he promptly became septic — and developed air under the right diaphragm as is shown on this abdominal roentgenogram. Exploration showed a large area of necrosis in the dome of the right lobe of the liver, as is shown on this slide. Postmortem showed thrombosis of the small branches of the right hepatic artery, as is seen in this photograph, and which is very similar to those described in Dr. Starzl's paper today. All of the major vascular anastomoses were patent.

This photomicrograph shows an area of relatively normal liver tissue with ductal proliferation in an area that was not infarcted. The area of transition between the infarct and the normal liver tissue is clearly seen. I would like to ask Dr. Starzl if he has an explanation for why arterial thrombosis occurs so late after grafting and if he believes there may be additional causative factors such as a manifestation of rejection? Do you

believe that adding arterial blood to the portal vein may be of any benefit in these patients? Would short- or long-term anticoagulation be of any help?

Dr. Thomas E. Starzl (Closing): I would like to thank Dr. Moore not only for the thoughts he expressed, but for the kindness he has displayed to us this afternoon and at all times in the past, which has made it possible for our two institutions to exchange data long before it was published.

The approach to preservation described by Dr. Fonkalsrud is a different one than we used in our cases. We were anxious to obtain longer term preservation than we have ever found possible with such simple perfusion methods. With our recently used technic we have been able to see the liver within the chamber and to evaluate it under circumstances of perfusion before anything was done to the recipient.

As to the cause for the late thrombosis, I think one can only speculate. It seems likely to us that the mechanical defect which I demonstrated earlier may not be completely occlusive and that there may be added factors, as Dr. Fonkalsrud suggested. Possibilities might include the swelling that occurs in liver homografts at the time of active rejection, or alternatively the diminished blood flow that is very often seen in the canine liver at the time of a potentially reversible rejection. With a combination of any of these circumstances, infant livers with their fragile arteries might be subject to a special risk of dearterialization.

On 2 May, 1968, Professor Roy Calne of Cambridge performed the first human liver transplantation after a preceding period of investigation of this operation in pigs (cf. Part I). Subsequent contributions of Calne and his associates at Cambridge and at King's College Hospital (London) are evident throughout this book.

Liver transplantation in man
I. Observations on technique and organization in five cases

British Medical Journal, 4: 535-40, 1968

R. Y. Calne and Roger Williams

Summary: In view of the extreme sensitivity of the human liver to ischaemic damage, the organization of clinical transplantation is of necessity complicated. From our preliminary experience of five human liver allografts we feel that active collaboration between hospitals is essential in order to practise human liver transplantation. It is unnecessary and undesirable to interfere in any way with potential liver donors. Nevertheless, the nature of the surgical technique requires that the liver is cooled within 15 minutes of death if satisfactory function is to result in the grafted organ.

This report describes technical difficulties that were encountered which can limit successful liver transplantation. The first patient was in severe liver failure and had an accessory liver graft in the splenic fossa after splenectomy. This liver suffered irreversible ischaemic damage, which led to an uncontrollable haemorrhagic state with exsanguination that resulted in death the day after operation. The second patient, a 10-month-old infant with biliary atresia and liver failure, died from cardiac arrest shortly after the operation.

The remaining three transplants developed good initial function. One patient survived 11 weeks, and one has returned to work.

Introduction

The liver can be transplanted to the normal anatomical position (orthotopic) or to an abnormal situation (heterotopic). A variety of heterotopic techniques have been described (Starzl *et al.*, 1966), but none is ideal. It is difficult to accommodate an extra liver in the abdomen in such a way that it receives arterial and portal blood and drains hepatic venous blood and bile. There is a danger that the multiple anastomoses will be kinked or otherwise compromised. Orthotopic liver transplantation requires preliminary hepatectomy of the recipient (Moore *et al.*, 1960; Starzl *et al.*, 1960). This provides the liver with normal anatomical surroundings, and revascularization and biliary drainage are straightforward.

Renal function can be replaced artificially by dialysis, which is used to support the patient before and if necessary after renal transplantation. There is no comparable method of replacing liver function. The two categories of fatal liver disease for which transplantation may be indicated are progressive liver failure with cirrhosis and primary malignant tumours of the liver. If the liver is malignant then hepatectomy is an obvious prerequisite, but with non-malignant progressive liver disease there are theoretical advantages in leaving the residual function of the patient's own liver and avoiding a dangerous and difficut hepatectomy. The unsatisfactory surgical features of heterotopic liver transplantation may, however, make this an unwise choice.

If the liver is left at 37° C. without a blood supply for more than 15 minutes serious damage is likely to occur. The liver cell necrosis results in impairment of function and also appears to be responsible for the uncontrollable haemorrhagic syndrome that occurred in our first case. To prevent this damage it is therefore essential to cool the liver within 15 minutes of death, by infusion of a chilled innocuous fluid through the portal vein. If this is followed by a cold plasma/bicarbonate/dextrose solution the liver can be kept at 4° C. with little deterioration for two hours (Schalm, 1968). Slow deterioration does, however, occur, and, since hepatectomy of the recipient can be an extremely difficult and time-consuming operation, longer preservation is desirable. The most satisfactory method so far described by Brettschneider *et al.* (1968) utilizes hyperbaric oxygen, hypothermia, and continuous perfusion with diluted blood. With this technique excellent preservation of canine livers for eight hours has been achieved, and an identical method has been used to preserve human livers for transplantation by Starzl *et al.* (1968a). Before laparotomy of the donor, while the preservation apparatus is being prepared, it may be helpful to cool the cadaver by means of a heart-lung machine with a refrigeration unit (Marchioro *et al.*, 1963).

Organization

It follows from the above remarks that the requirements for a clinical liver transplantation are as follows: a recipient with fatal liver disease at a terminal stage, but who is nevertheless fit enough to withstand a major and prolonged operation, and a donor whose liver can be cooled within 15 minutes of death. It is not surprising that clinical liver transplantation has been slow in developing. Of five heterotopic liver transplants in man the longest survival was 35 days (Starzl *et al.*, 1967). Previous to July 1967 nine orthotopic liver transplants were performed in man and the longest

survival was 23 days. Since that time the clinical liver transplantation programme at Denver has had more sucess (Starzl *et al.*, 1968a, 1968b). Of 17 transplants in 16 patients the longest survivor was more than a year, the patient eventually dying from secondary deposits of his original primary hepatoma. Of the seven patients surviving at the present time, one has received a second liver transplant after the first had been rejected and removed (Starzl, Brettschneider, and Porter, personal communication).

In view of the major organizational requirements for a successful liver transfer it is necessary for there to be a period of warning during which arrangements can be made. The recipient should therefore have sufficient residual liver function to permit him tc wait for the availability of a transplant. The most suitable donors are cases in which resuscitation has been performed, but where irreversible brain damage has occurred and a decision has been made, quite irrespective of any transplantation considerations, to cease resuscitation. When recovery is impossible it is customary to cease artificial ventilation and support of the circulation. This was true before the development of organ transplantation, and we feel it is unnecessary to modify the procedure in any way for the purposes of transplantation. The medical staff caring for the patient should not be involved in the transplantation operation. After resuscitation has been abandoned no procedures are started on the donor until it is clear that there is no spontaneous respiration or heart beat, and death has been confirmed by independent medical staff. Liver transplantation can then be performed entirely within the confines of long-established ethical medical treatment. All that is necessary is for the doctors who have decided to stop rescuscitation to inform the transplantation team, who will then be prepared.

Since the availability of suitable donors in any one institution is limited, it is felt that if clinical liver transplantation is to be developed in the United Kingdom it will be necessary for hospitals to collaborate. We do not feel justified in moving a potential donor from one hospital to another for the purposes of organ transplantation. Instead, we move the transplant team and the recipient to the same hospital as the donor. A close liaison has been established between Addenbrooke's Hospital, Cambridge, and King's College Hospital, London, where there are complementary interests in liver transplantation and liver disease. It is proposed to undertake a definitive programme of liver transplantation, collaborating with other hospitals where this is possible.

This report summarizes certain technical aspects of our first five cases. The full clinical details of Cases 3 and 4 are given in an accompanying article at p. 541 (Calne et al., 1968). Reports of the anaesthesia and tissue typing are in preparation.

Case 1

A 47-year-old man was admitted to Addenbrooke's Hospital on 23 May 1967 with gastrointestinal bleeding. He had a past history of alcoholism which had led to liver failure, with ascites and periods of coma.

Fig. 1.— Diagram of operative procedure in Case 1, showing heterotopic liver allograft in the splenic bed.

On examination he was pale, jaundiced, and ill-looking. The liver was not palpable, but the spleen could be felt below the left costal margin. There was marked ascites. Haemoglobin was 11.3 g./100 ml., white blood cell count 5,400/cu.mm., platelets 60,000/cu.mm., prothrombin ratio 17-13, and bilirubin 2 mg./100 ml. (1.6 mg./100 ml. conjugated). Splenoportogram showed large gastro-oesophageal varices. Serum albumin varied between 1.9 and 2.3 g./100 ml. The patient had had two previous attacks of haematemesis and melaena requiring transfusion and had been comatose for one week. He recovered from coma and became fully alert. In view of his continued gastrointestinal bleeding, low albumin, clinical jaundice, and a previous history of coma, it was felt that any surgical procedure aimed at reducing portal venous pressure would result in irreversible coma. His liver disease was considered to be beyond conventional therapy. The possibility of hepatic transplantation was discussed with the patient, who was anxious that this should be tried.

The donor was a 50-year-old woman who died from a head injury. She was brought into hospital dead and attempts at resuscitation were made for half an hour. There was no response to the resuscitative efforts.

Via a midline abdominal incision the superior mesenteric vein was cannulated and perfused with 1 litre of chilled balanced salt solution. An incision was made in the right common iliac vein to allow egress of perfusate. While perfusion was in progress the liver was removed and put into a plastic bag surrounded by cold perfusion fluid. The hepatic artery was similarly perfused and the plastic bag was surrounded with ice. This procedure was used in the four subsequent cases.

Before the start of perfusion there had been a period of one hour in whch the liver at 37° C. had been unperfused.

Recipient Operation

Under light general anaesthesia a left subcostal incision was made and extended across the midline. Ascitic fluid was aspirated. The spleen, which was extremely large, was removed. The liver was taken out of the plastic bag at 11.30 a.m., three hours after the death of the donor. It was rotated so that the hilus faced towards the midline and it was placed in the splenic fossa, the shrunken cirrhotic liver allowing room for the transplant. The gall bladder faced anteriorly and the suprahepatic inferior vena cava faced towards the renal vein. The anastomoses were performed as shown in Fig. 1. The coeliac artery was anastomosed end-to-end to the splenic; the superior mesenteric vein was anastomosed end-to-end to the splenic vein, and the splenic vein of the transplant was anastomosed to an accessory splenic vein, which lay in the tail of the pancreas. Clamps were removed from the splenic vessels and blood was allowed to perfuse the liver. The infrahepatic inferor vena cava was ligated. The suprahepatic inferior vena cava was anastomosed end-to-side to the left renal vein. The liver appeared to perfuse satisfactorily. The common bile duct was ligated and the gall bladder was anastomosed to the end of a Roux loop of jejunum. The anastomoses took an hour, so that the total ischaemia time was four hours. The wound was closed in layers with drainage. Though no heparin had been given after operation there was a continuous steady loss of blood from the drains. Two hours after the operation bleeding became more profuse; blood oozed from the wound and around the intravenous infusion. The blood clotted and then underwent complete fibrinolysis after about 20 minutes. The patient did not recover consciousness.

The wound was reopened and bleeding was seen to be coming from the whole operative site and particularly from the caval anastomosis, where additional sutures were inserted. Haemorrahge continued in spite of the administration of aminocaproic acid, intravenous fibrinogen, and fresh blood. In all, 23 litres of blood were transfused. The patient died at 5.30 next morning.

The biopsy taken shortly after transplantation of the liver showed marked degenerative changes in many of the liver cells (Special Plate, Fig. 2). Necropsy revealed the typical changes of severe portal cirrhosis of the patient's own liver with large gastro-oesophageal varices. The transplanted liver was severely congested. The anastomoses were all patent, though the inferior vena caval anastomosis was narrowed by the additional sutures. Histology of the transplant showed congestion, haemorrhage, and areas of liver necrosis. Some liver cells were reasonably well preserved. There was no regular zonal distribution of these changes.

Comment.— This liver was removed from a cadaver after irreversible ischaemic damage had occurred, and this led to an uncontrollable haemorrhagic state. The heterotopic situation of the liver was not

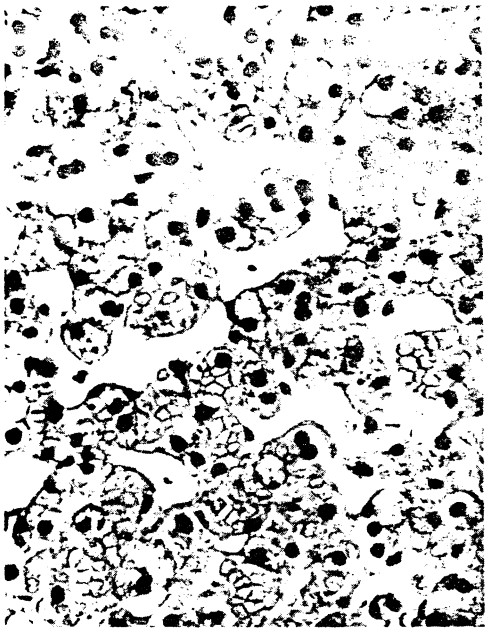

Fig. 2.— Biopsy of liver allograft in Case 1, showing degeneration of liver cells and intrahepatic haemorrhage. (x 335.)

satisfactory and congestion of the liver at necropsy indicated poor venous drainage.

Case 2

A child of 10 months was admitted to Addenbrooke's Hospital on 11 June 1968 with jaundice which had developed shortly after birth. Laparotomy at the age of 2 months confirmed the diagnosis of biliary atresia. During the two months preceding admission the child had developed biliary cirrhosis with ascites and was considered to be unsuitable for any further treatment.

Haemoglobin was 8.6/100 ml., bilirubin 8.2 mg./100 ml. (5.7 mg. conjugated), total protein 6.2 g./100 ml. with a moderate increase in alpha2 - and gamma-globulin.

On examination he was extremely jaundiced, with enormous abdominal distension, dilated superficial veins coursing over the anterior abdominal wall, and gross ascites. The liver was markedly enlarged. The spleen was just palpable. An umbilical hernia was present.

It was felt that the prognosis was hopeless, and the parents requested that an attempt should be made to perform a liver transplant.

The donor was a child of 2 who had died as a result of upper respiratory tract infection which led to cardiac and respiratory arrest. The time of death was 4.35 p.m. Cardiac massage and artificial ventilation were attempted without any result. At 4.57 p.m. a transverse upper abdominal incision was made, a cannula was inserted into the superior mesenteric vein, and the liver was perfused with 1 liter of cold Hartmann's solution containing 2,000 i.u. of heparin and then with 500 ml. of plasma/bicarbonate/glucose solution[1] (Schalm, 1968). An incision was made into the inferor vena cava to allow the perfusate to escape. An accessory right hepatic artery was found to be arising from the superior mesenteric artery; this was preserved together with the other vessels and the bile ducts.

Recipient Operation

A bilateral subcostal incision was made. Dilated anastomotic veins in the anterior abdominal wall were individually ligated and divided. Ascitic fluid was aspirated. Dense adhesions between the bowel, the parietes, and the liver were divided. The liver was huge, green, and embossed with bile cysts; there were large lymph nodes at the hilus. Removal of the diseased liver was time-consuming. The liver was skeletonized until it was attached only by its vascular connexions. The portal vein and inferior vena cava were temporarily occluded, and since this produced no deterioration in the general condition of the patient no vascular shunts were used. Hepatectomy was completed at 7.35 p.m., when the donor liver was removed from ice.

The vena cava was anastomosed to the vena cava above the liver, and the portal vein was anastomosed to the portal vein end-to-end. The clamp was removed from the portal vein at 7.55 p.m. and blood was allowed to perfuse the liver. After 10 ml. of blood had been discharged from the inferior vena cava below the liver this vessel was clamped and the vascular clamp was removed from the inferior vena cava above the liver. The aorta was mobilized and cross-clamped, a Carrel patch with the coeliac artery of

[1] Preservation fluid containing 400 ml. of reconstituted human plasma, 10 ml. of 2% procaine, 8 ml. of 5% glucose, and 20 ml. of 1.4% sodium bicarbonate.

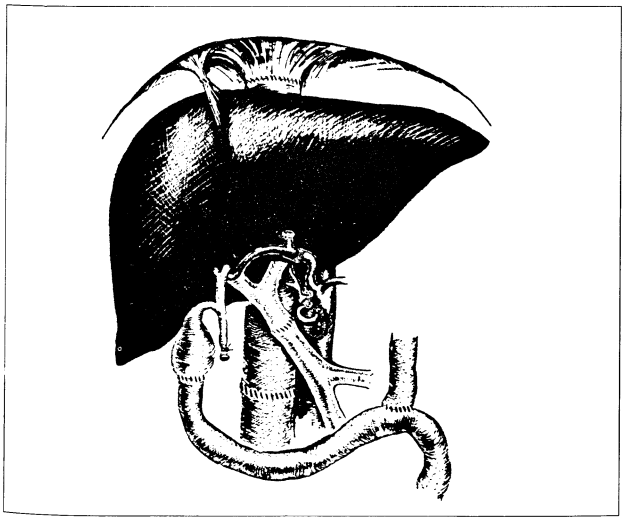

Fig. 3.— Diagram of operative procedure in Case 2.

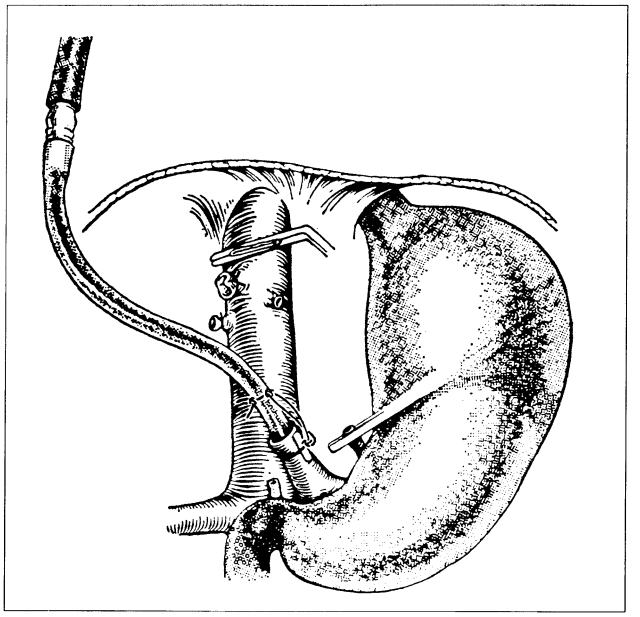

Fig. 4.— Diagram of the operation at the stage of hepatectomy in Case 3, showing the hepatic veins clamped and the inferior vena cava intact. A direct shunt emerges from the portal vein and drains into the right internal jugular vein.

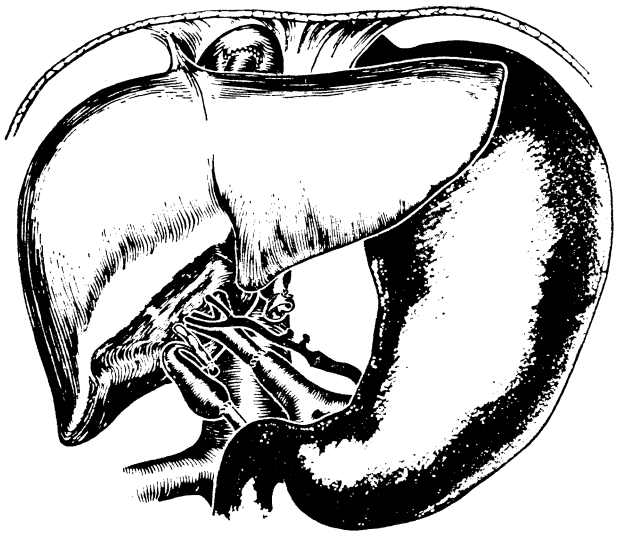

Fig. 5.— Diagram of operative procedure in Case 3, showing anastomosis of the suprahepatic inferior vena cava to the hepatic veins. The infrahepatic vena cava is ligated.

the donor was anastomosed end-to-side to the aorta of the recipient. The superior mesenteric artery of the donor was anastomosed end-to-end to the recipient's hepatic artery and the inferior vena cava was anastomosed end-to-end to the inferior vena cava below the liver. All anastomoses were completed by 8.50 p.m. The bile duct was ligated and the gall bladder was anastomosed to a Roux loop of jejunum (Fig. 3). The liver was rather pale and blotchy and did not appear to be perfusing well, but all anastomoses looked satisfactory. The falciform ligament of the transplant was sutured to that of the recipient. No heparin was used, 4 mg. of fibrinogen was given intravenously, haemostasis was achieved, and the wound was closed in layers. Intravenous dextrose and bicarbonate were given.

The child's condition improved at the end of the operation; he was breathing spontaneously and regained consciousness. Half an hour after the end of the operation there was a sudden cardiac and respiratory arrest, the cause of which was not determined. Attempts at rescuscitation failed. At necropsy no cause of death could be found. All anastomoses of the transplant were patent. Microscopy of the transplant showed slight centrilobular necrosis of many lobules and oedema of the portal tracts with

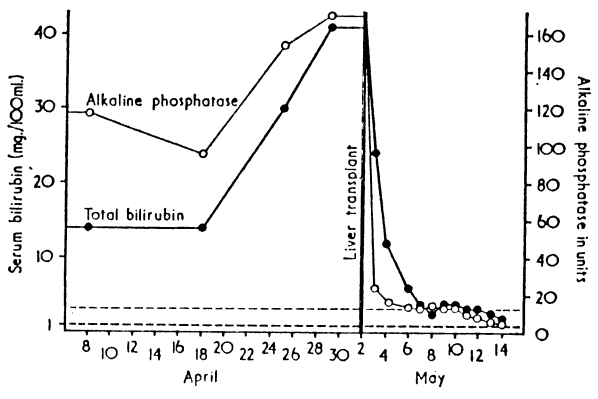

Fig. 6.— Chart of alkaline phosphatase in King-Armstrong units and total bilirubin in mg./100 ml. of blood (Case 3). Blood levels of both fell very rapidly after liver allografting on 2 May 1968.

dilatation of lymphatics. There was a slight neutrophil infiltrate of the hepatic capsule. Most of the liver parenchyma was well preserved.

Comment.— It was felt that the extensive operative procedure in an exceedingly ill child was too great for it to withstand, but the liver preservation appeared to be adequate.

Case 3

A 46-year-old woman was admitted to Addenbrooke's Hospital on 7 February 1968 with a primary cholangiocarcinoma which had caused obstructive jaundice and gastrointestinal haemorrhage. Chlorambucil treatment for her tumour resulted in marrow depression and septicaemia. Full details are reported elsewhere (Calne *et al.*, 1968).

Though in an extremely ill state, the patient was most anxious to have a liver transplant, and in spite of persisting marrow damage liver transplantation was performed.

The donor was a 5-year-old child who had died as a result of mumps encephalitis. Death occurred at 4.50 p.m. A transverse abdominal incision was made and the superior mesenteric vein was perfused with cold heparinized Ringer's solution at 5.05 p.m. An incision was made in the right common iliac vein to allow egress of perfusate. The perfusate was changed to plasma bicarbonate dextrose solution. Similar perfusate was used in the hepatic artery.

Recipient Operation

A bilateral subcostal incision was made. The liver was found to be huge, with deposits of growth palpable in both lobes but no ascites and no evidence of growth outside the liver. There were dense vascular adhesions between the porta hepatis, omentum, and duodenum. The liver was skeletonized until it was attached only by its vascular connexions. During the dissection the patient's blood pressure tended to fall, probably owing to manipulation of the liver causing embarrassment of respiration and cardiac action and intermittent obstruction of the vena cava. The patient was systematically heparinized with 10,000 units of heparin. The portal vein was clamped at 6.08 p.m. and portal venous blood was shunted to the right internal jugular vein (Fig. 4). It was felt that the inferior vena cava of the recipient was much too large to be anastomosed to the vena cava of the small donor liver; therefore the liver was filleted off the vena cava, clamping the main hepatic vein and ligating all other branches. The malignant liver was removed at 6.20 p.m. and the donor liver was removed from the ice at 6.40 p.m.

The suprahepatic inferior vena cava was anastomosed end-to-end to the main hepatic vein. The portal vein was anastomosed end-to-end to the portal. There was considerable discrepancy in size, and plication was necessary in order to complete the anastomosis. The clamp was removed from the portal vein at 7.11 p.m. and blood was allowed to perfuse the liver. After 30 ml. of blood had been discharged from the inferior vena cava below the liver this vessel was ligated and the clamp on the hepatic vein was removed. The coeliac artery with a rim of aorta was anastomosed end-to-end to the divided hepatic artery of the recipient (Fig. 5). This anastomosis was completed at 7.23 p.m., and on release of the arterial clamp an excellent pulse was felt throughtout the length of the hepatic artery of the donor and the liver changed from a dark red colour to bright salmon pink with a normal consistency. The diameter of the common bile duct of the recipient was 0.7 cm., while that of the donor was 0.3 cm. An end-to-end anastomosis was felt to be surgically unsatisfactory. The alternative of anastomosing the gall bladder to the bowel was felt to be undesirable because of the danger of ascending cholangitis. Accordingly the gall bladder was mobilized from its bed.

The distal two-thirds were freed from the liver and turned downwards. There was no acute kink and the fundus of the gall bladder was anastomosed to the open end of the common bile duct with continuous 4/0 chromic catgut. The anastomosis lay in a satisfactory position, and bile was seen to be produced from the liver at this stage. The common bile duct was then ligated. The falciform ligament was sutured to the diaphragm, and a minute liver biopsy was taken. Yates drains were brought out of the lateral extremities of the wound, which was closed in layers. Every attempt was made to secure haemostasis, but the whole of the operation site was oozing blood at the end of the operation and the patient felt cold. The heparin was reversed with protamine. Fibrinogen was given, and the patient was warmed with blankets. Significant bleeding from the wound ceased in the subsequent two hours. During the operation the patient received 7 litres of

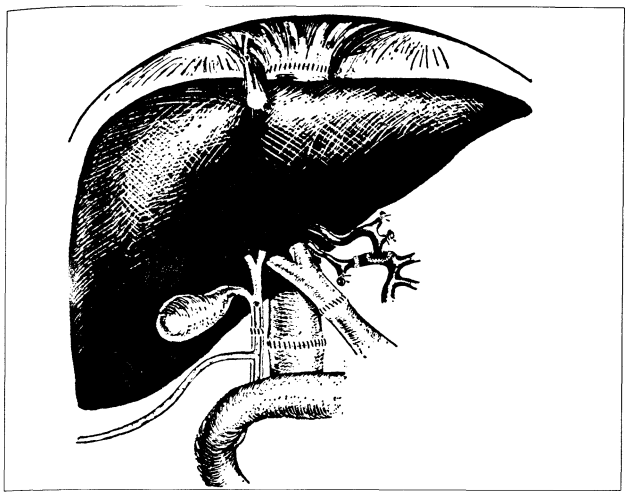

Fig. 7.— Operative procedure in Case 4. The long arm of the T tube emerges through the recipient's common bile duct.

blood, 100 ml. of 20% mannitol, and intravenous dextrose and bicarbonate solutions.

Biopsy of the transplanted liver shortly after revascularization showed well-preserved liver cells. Initial function of the liver was considered to be good (Fig. 6). The patient rapidly regained consciousness. Full operative details are recorded elsewhere (Calne *et al.*, 1968).

The patient died 11 weeks after operation following partial infarction of the liver. This appeared to be caused by a clot arising in the recipient hepatic artery, where it had been clamped during the operation.

Case 4

A 41-year-old man was admitted to King's College Hospital on 23 September 1968 with a primary liver cell cancer. Full details are reported elsewhere (Calne *et al.*, 1968). There was massive enlargement of the liver, but the patient was not jaundiced and was ambulant. In view of the hopeless prognosis he was anxious to have a liver transplant.

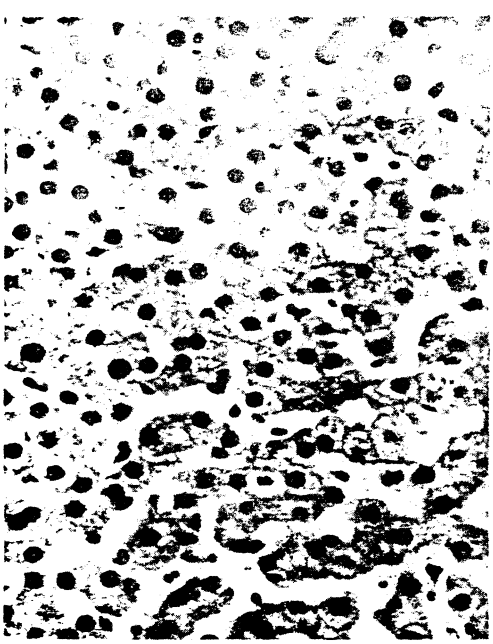

Fig. 8.— Operative biopsy of liver allograft of Case 4 at the time of transplantation, showing well-preserved liver parenchyma. (H. and E. x 335.)

The donor was a 13-year-old child who had died as a result of a head injury. Death occurred at 11.27 p.m. A bilateral subcostal incision was made, and the superior mesenteric vein was perfused with 2 liters of cold heparinized Hartmann's solution followed by 200 ml. of plasma bicarbonate dextrose solution. The cold infusion began at 11.40 p.m. An incision was made in the right common iliac vein to allow egress of perfusate. An accessory right hepatic artery was found to be arising from the superior mesenteric artery. This was preserved together with the other vessels and the bile ducts. The liver was immersed in cold saline in a bowl surrounded with ice at 12.35 a.m.

Recipient Operation

A bilateral subcostal incision was made. The liver was grossly enlarged, and there were numerous vascular adhesions between the liver, parietes, and surrounding viscera. These were ligated and divided. Huge veins were coursing over the surface of the liver, which bled on touch. During mobilization of the liver bleeding occurred whenever the venous outflow was obstructed. There was no evidence of extrahepatic spread of tumour, but the whole of the left lobe of the liver was replaced by tumour and multiple small tumour deposits were scattered through the right lobe. During the final stages of the skeletonization of the liver the infrahepatic vena cava was divided; both ends were controlled and ligated. The patient's systolic blood pressure fell to 60 mm. Hg, but gradually rose with replacement of blood. The hepatectomy was completed at 1.00 a.m., when the donor liver was removed from the ice. The vena cava was anastomosed to the vena cava above the liver; the portal vein was anastomosed to the portal vein end-to-end. The clamp was removed from the portal vein at 1.26 a.m., and blood was allowed to perfuse the liver. After 30 ml. of blood had been discharged from the inferior vena cava below the liver this vessel was clamped and the suprahepatic caval clamp was removed. The liver rapidly assumed a satisfactory colour of pale pink and normal consistency. The coeliac artery was anastomosed end-to-side to the proximal hepatic artery of the recipient, a Carrel patch being used. The donor superior mesenteric artery was anastomosed end-to-end to the cut distal hepatic artery (Fig. 7). The clamp was removed from the hepatic artery at 1.55 a.m., and the infrahepatic vena caval anastomosis was made end-to-end.

The common bile duct was anastomosed with 4/0 interrupted catgut sutures end-to-end over the upper limb of a rubber T-tube, which was brought out through the recipient's common duct below the anastomosis. Bile was seen to be produced by the liver at this stage. The T-tube was brought out through a stab incision. Haemostasis was achieved, the falciform ligament was sutured to the diaphragm, and a minute liver biopsy was taken. Two grammes of ampicillin dissolved in 50 ml. of saline was

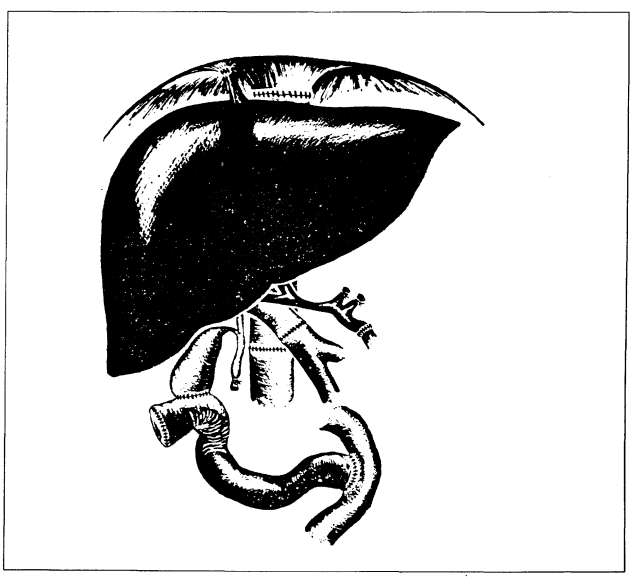

Fig. 9.— Diagram of operative procedure in Case 5.

introduced under the diaphragm and in the subhepatic space. Yates drains were brought out by stab incisions through both flanks from both subdiaphragmatic areas and from the subhepatic space of the right side. The wound was closed in layers.

The patient's general condition at the end of the operation was good and he woke immediately from the anaesthetic, but continuous haemorrhage occurred from the drainage site on the right side, and after 12 hours the abdomen was re-explored. One litre of clotted blood was found in the peritoneal cavity; this was removed. No bleeding could be found at any point in relation to the liver or elsewhere. The wound was closed, and haemorrhage was then seen to be coming from the drain site in the right flank. This wound was opened further, and an artery in the subcutaneous tissue was found to be bleeding; this was oversewn, and other small vessels near by were clamped. Bleeding then ceased, and the patient's general condition became excellent. He had received mannitol, bicarbonate, dextrose, and 22 litres of blood. The transplant biopsy taken shortly after revascularization showed well-preserved liver parenchyma (Special Plate, Fig. 8).

The subsequent postoperative course is recorded elsewhere (Calne *et al.*, 1968). At the time of writing the patient is clinically well and has returned to work, having been discharged from hospital on the seventeenth postoperative day.

Case 5

A 46-year-old man was admitted to King's College Hospital on 27 October 1968 with a primary cholangiocarcinoma which had caused obstructive jaundice. In view of the hopeless prognosis he was anxious to have a liver transplanted.

The donor was a 64-year-old man who had died as a result of a head injury. Death occurred at 10.26 p.m. A bilateral subcostal incision was made, and the superior mesenteric vein was perfused with 2 litres of cold heparinized Hartmann's solution followed by 200 ml. of plasma/bicarbonate/dextrose solution. Cold infusion began at 10.30 p.m. An incision was made in the right common iliac vein to allow egress of perfusate. The liver was removed and placed in cold saline at 11.15 p.m.

Recipient Operation

A bilateral subcostal incision was made. The liver was grossly enlarged, but there was no evidence of growth on the surface of the liver or elsewhere in the peritoneal cavity. There were dense adhesions in the region of the aditus. The common bile duct was extremely fibrotic, and it was felt that it would be unsuitable for anastomosis. It was therefore divided and ligated just above the duodenum. Some purulent material discharged form the hilus of the liver; this was cultured. Hepatectomy was completed at 11.15 p.m., when the donor liver was removed from the ice.

The vena cava was anastomosed to the vena cava above the liver, and the portal vein was anastomosed to the portal vein end-to-end and blood was allowed to perfuse the liver. After 30 ml. of blood had been discharged from the inferior vena cava below the liver the vessel was clamped and the suprahepatic vena caval clamp was removed. The liver rapidly assumed a satisfactory colour of pale pink and normal consistency. The coeliac artery of the donor was anastomosed to the hepatic artery of the recipient end-to-end. The clamp was removed from the hepatic artery at 12.15 a.m., and there was an excellent arterial pulse up to the hilus of the liver, with no evidence of kinking or obstruction (Fig. 9). The infrahepatic vena caval anastomosis to a Roux loop of jejunum which was brought up through the mesocolon. There was considerable bleeding from the jejunal wall in spite of the fact that diathermy had been used to open it, but it appeared that the all-coats catgut layer had stopped the bleeding. Two grammes of ampicillin dissolved in 50 ml. of saline was introduced under the diaphragm and in the subhepatic space. A Yates drain was brought out through the right extremity of the main wound, which was closed in layers.

The patient's condition at the end of the operation was good, and he rapidly regained consciousness. The following day there was severe gastrointestinal haemorrhage, which continued in spite of normal clotting factors and transfusion of fresh blood. Laparotomy was therefore performed, and the bleeding was found to be coming from the jejunal side of the cholecystduodenostomy. The bleeding points were individually ligated and the anastomosis was redone. The transplant biopsy taken shortly after revascularization showed a well-preserved liver. He received, in all, 7 litres of blood, 4 g. of fibrinogen, and 200 mg. of hydrocortisone in addition of mannitol, bicarbonate, and dextrose. No heparin or protamine was given. The postoperative course was complicated by further gastrointestinal haemorrhage, jaundice, and peritonitis. Death from sepsis occurred after three weeks.

Discussion

These five cases allow several conclusions to be made. The irreversible ischaemic damage in Case 1 leading to an uncontrollable haemorrhagic syndrome reinforces the importance of reducing the warm ischaemic time to a minimum of 15 minutes between death of the donor and cooling of the liver. In the subsequent four cases the donor liver was cooled within 15 minutes of death, and in Cases 3-5 with total ischaemic times (from death of donor until revascularization with portal blood) of 141, 106, and 94 minutes, excellent preservation was achieved by the simple cooling that was used (Schalm, 1968).

The extremely serious liver failure in Case 1 by the time the transplantation was performed made the likelihood of success unlikely even with a well-preserved and satisfactorily transplanted heterotopic liver. Though the splenic fossa provided room for the liver transplant, and the anastomoses appeared satisfactory at the time of the operation, the venous drainage was inadequate, and the other anastomoses might have been compromised if the patient had adopted an upright posture. We feel that the orthotopic site is preferable.

In Case 1, 2, and 5 biliary drainage was to a Roux loop of jejunum. This was felt to be preferable to cholecystduodenostomy, though less desirable than biliary drainage, which retains the patient's own sphincter of Oddi, as in Cases 3 and 4.

The cause of death in Case 2 was not apparent; however, the child was extremely frail and ill, and athe recent rapid development of ascites indicated terminal liver failure.

In Case 3 there were technical difficulties in fitting a small child's liver into an adult. The infarction of the transplant, however, was not due to the discrepancy in size, since the arterial thrombus arose on the recipient hepatic artery where the vascular clamp had been applied. The cholecystocholedochostomy appeared satisfactory and well vascularized at the time of operation.

One patient (Case 4) was less ill than the others. The hepatectomy was particularly difficult, but he withstood the operation extremely well.

We are in full agreement with Starzl et al. (1968b) that in humans it is unnecessary to shunt blood from the inferior caval and portal systems when the patient is anhepatic. It is, however, desirable to have two intravenous infusions set up in the superior vena caval system before clamping the inferior vena cava, so that the return of blood to the heart can be augmented should there be a precipitous fall in blood pressure.

At present we believe the most suitable cases for liver transplantation are patients with primary hepatic or biliary tract carcinoma and biliary atresia. Some idea of the number of such cases present in this country was obtained by Terblanche and Riddell (1967) from an analysis of the Registrar General's Statistical Review of England and Wales for the year 1964. There were 429 deaths from primary hepatic carcinoma and 1,070 deaths from primary biliary carcinoma (gall bladder and bile duct) for all age groups, with corresponding figures for those dying between 0 and 60 years of 145 and 195. Orthotopic transplantation of the liver is a major surgical procedure, and it is unlikely that patients over the age of 60 will often be considered, in view of the high incidence of associated disease, particularly of the cardiovascular system. About 30% of primary hepatic tumours may have distant metastases by the time of diagnosis, and perhaps another third of such patients are treatable by hepatic lobectomy. Carcinomas of the hepatic and common bile duct are often slowly growing and metastasize late, so that about 130 to 160 patients from these two groups may be suitable for hepatic transplantation each year.

Biliary atresia, though rare, is another condition with an almost uniformly poor prognosis, in which the defect can rarely be corrected surgically. In the same year in th 0-60 age group there were 490 deaths from cirrhosis, 106 from acute viral hepatitis, and 54 from acute hepatic failure due to other causes, including drugs. All these are potential recipients, but the metabolic derangements of hepatic coma, the portal hypertension, and the frequently attendant renal impairment usually present in these patients are likely to increase the operative and immediate postoperative mortality considerably. Operating on patients with acute viral hepatitis may also carry grave dangers to the surgical and nursing teams.

With regard to the availability of donors in 1964 there were 1,135

deaths from subarachnoid haemorrhage and primary brain tumour and 2,478 from motor-vehicle accidents with a fractured skull or head injury in the 0-45 age group (Terblanche and Riddell, 1967). In both these groups some of the patients are likely to be maintained on respirators until the final demise. The patients dying in the older age groups carry a higher risk of their livers containing an occult secondary carcinoma. Despite an apparent adequate supply of donors, there will be major problems in organization for the individual patient, as already outlined. These problems will persist until organ preservation techniques have been developed, which will enable the donor liver to be preserved for periods of more than two to three hours.

Though the indications for liver transplantation will be widened in the future, at the present time the most suitable cases are adults with fatal hepatic disease who are too ill for an independent existence outside hospital, but have not yet reached a moribund stage.

We wish to thank our medical, nursing, and technical colleagues who have helped to initate this transplantation programme. We have had most generous assistance from so many people that it would be invidious to mention individual names. We are particularly indebted to the anaesthetics, bacteriological, biochemical, pathological, haematological, radiological, and medical illustration departments of Addenbrooke's Hospital and King's College Hospital. We thank Mrs. M. Allen for preparing the drawings.

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Dr. Gerard Martineau

Until the publication of this article, there was virtually no mention of biliary complications after liver transplantation. The four reported cases were tragic since the obstructions had been neglected until irreversible inspissated bile concretions formed. Diagnosis of such complications by transhepatic cholangiography was not attempted commonly because of the dangers and deficiencies of this technique, but this all was to change shortly with the development in Japan of the so-called Chiba ("skinny") needle. From this time onward, biliary obstruction or other complications of bile duct obstruction were high on the list of diagnostic possibilities if postoperative hepatic dysfunction developed. The flawed procedure of cholecystoduodenostomy was not yet abandoned, but this step soon was to follow.

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Delayed biliary duct obstruction after orthotopic liver transplantation

Surgery, 72: 604-10, 1972

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After orthotopic liver transplantation and bilary reconstruction by cholecystoduodenostomy, four of 40 patients developed delayed obstruction of the cystic duct. The recipients had the clinical syndrome of fulminating cholangitis with jaundice, fever, leukocytosis, toxemia, and bacteremia. All four patients died; of the four, two patients died despite late reoperation and re-establishment of bile drainage by choledochoenterostomy. In all four cases, a factor contributing to the biliary obstruction may have been infection of the extrahepatic biliary ducts with or without ulceration, and in three of the livers, there was evidence of infection of the ducts with CMV. If cholecystoduodenostomy is used in future cases, prompt re-exploration and conversion to choledochoenterostomy should be considered if the diagnosis of duct obstruction, cholangitis, and persistent bacteremia are made.

In our institution biliary duct reconstruction after orthotopic liver transplantation has usually been employed with cholecystoduodenostomy after ligation of the graft distal common duct.[4] After this type of reconstruction, chronic survival of both experimental animals and man has been achieved. However, the present communication will present four examples of failure of cholecystoduodenostomy resulting from delayed obstruction of the cystic duct near its junction with the common duct. In each instance, the result was death. Evidence will be presented that cytomegalovirus (CMV) infection of the extrahepatic biliary duct system may have contributed to these complications.

METHODS

The four patients underwent the liver replacement operation depicted in Fig. 1, *A*, including cholecystoduodenostomy. They were all treated with triple-drug regimen that included horse antilymphocyte globulin (ALG) and prednisone. The third agent for Patients 1 and 2 was azathioprine,[4] and Patients 3 and 4 were treated instead with cyclophosphamide.[5]

The patients who developed cystic duct obstruction were aged 16 months, 14 years, 16 years, and 11 years. Their primary diagnoses were biliary atresia (Patients 1 and 3) and chronic aggressive hepatitis (Patients 2 and 4).

RESULTS

Incidence of the complication. The four cystic duct obstructions occurred in the first 44 consecutive liver transplantations. Since cholecystoduodenostomy had been performed in only 40 of the 44 recipients, the corrected incidence was 10 per cent.

The clinical syndrome. At the time of the original transplantation, all four homografts secreted bile which welled up in the gallbladder through the cystic duct. Good hepatic function with clearing of preexisting jaundice continued during the early convalescence. (Fig. 2)

After 3 to 22 days, hyperbilirubinemia recurred, preceded by major increases in the alkaline phosphatase and accompanied by minor rises in the transaminases (Fig. 2). In each of the four cases, rejection was thought to be present and was treated with increased doses of prednisone (Fig. 2). In three of the four instances, there was some remission of the secondary jaundice (Fig 2). This was initially interpreted as support for the diagnosis of rejection.

All of the patients became remittently febrile with the onset of liver function aberrations. Shortly after, bacteremia was detected in multiple blood cultures (Fig. 2). The microorganisms were those indigenous to the gastrointestinal tract, being predominantly gram-negative pathogens (Table I). Once the bacteria appeared in the bloodstream, blood cultures remained positive until death in spite of antimicrobial therapy. The patients appeared toxic with marked tachycardia accompanying the fever spikes. Leukocytosis (as high as 50,000 per cubic millimeter) and thrombocytopenia (as low as 5,000 per cubic millimeter) were invariably noted (Fig. 2).

Each patient had several liver scans. In Patient 1, partial gangrene of the right hepatic lobe occurred after thrombosis of the right hepatic artery; the necrotic area did not pick up the technetium isotope. The other three

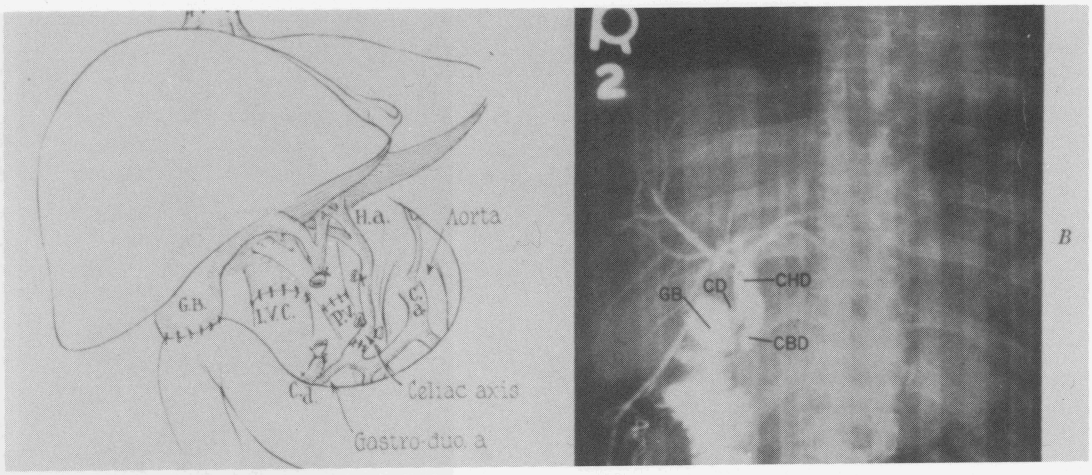

Fig. 1.— Biliary duct reconstruction with cholecystoduodenostomy. A, Surgical technique. B, Operative cholangiogram in a patient who developed jaundice almost three months after orthotopic transplantation. In this case, obstruction was ruled out.

Table I. *Clinical features of four patients with posttransplantation biliary obstruction*

Case No.	Postoperative jaundice recurrence (days after transplantation)	Maximum secondary bilirubin rise (mg. %)	Remission of secondary jaundice	Bacteremia	Other infections	Postoperative survival (days)
1	3	22	to 3 mg. %	Aerobacter-Klebsiella; *E. coli*	Pneumonitis	105
2	22	21.5	to 6 mg. %	*E. coli*	Pneumonitis	63
3	15	18	to 2.5 mg. %	*E. coli*	None	46
4	5	14.5	None	*E. coli; Clostridium perfringens*	None	31

homografts had minor abnormalities but not in any distinctive pattern.

Efforts at surgical correction. In Patients 1 and 3, a re-exploratory operation was performed. By insertion of a ballon catheter into the gallbladder (Fig. 3, *A* and *B*), the duct system was re-evaluated. Although the gallbladder and cystic duct were collapsed, the common duct and the intrahepatic ducts were dilated (Fig. 3, *C*). The liver had multiple soft areas which proved upon aspiration to contain a bacteria-laden thin fluid. The gallbladder was removed and choledochoduodenostomy was performed. After operation, there were falls in the bilirubin (Fig. 2), but both patients died of uncontrolled sepsis.

Pathologic observations. At autopsy from 31 to 105 days after transplantation, there was a chalklike, crumbly debris which formed casts in some of the dilated intrahepatic ducts of all four livers (Table II). The cystic ducts were small in Patients 1 and 4, similar to the findings previously demonstrated at reoperation in Patients 2 and 3.

Patient 1 had a complete thrombosis of the right hepatic artery with consequent gangrene of the right lobe of the liver (Table II). In Patient 4, an abscess of the right hepatic lobe, which was in free communication with the dilated right main hepatic duct, had ruptured through the liver into the subphrenic space.

At the time of reoperation or at autopsy, bits of gallbladder were obtained. Histopathological evidence of chronic cholecystitis was found in three of the four gallbladders (Table II). There was widespread ulceration of the lining epithelium in Patients 1 and 4 and focal ulceration in Patient 3. These three gallbladders also showed infiltration of the submucosa by mononuclear cells and polymorphonuclear leukocytes and replacement of the muscle by fibrous tissue. Intranuclear inclusions resembling those produced by cytomegalovirus (CMV) were present in many of the mucosal epithelial, submucosal epithelial, and connective tissue cells of the cystic ducts and gallbladders from Patients 2, 3, and 4 (Fig. 4). Groups of enlarged virus-infected cells that had been shred were present on the gallbladder and duct mucosal surfaces and in the lumens.

The liver homografts from Patients 1, 3, and 4 showed clear evidence of large biliary duct obstruction. The portal tracts were enlarged and were lightly infiltrated with mononuclear cells and polymorphonuclear leukocytes. There was proliferation of small bile ducts and ductules. The ducts were dilated and some contained inspissated bile. Bile lakes were present in Patients 1 and 3. Centrilobular cholestasis was marked in Patient 1 and slight in Patients 2 and 3. There was marked chronic cholangitis in Patients 3 and 4. Cells with CMV-like intranuclear inclusions were present in the hepatic homografts of Patients 2, 3, and 4. None of the homografts showed clear evidence of past or active rejection, although IgM and Clq were present in some of the vein walls of the graft in Patient 1.

Many cytomegalic inclusion cells were present in the alveolar epithelium of the lungs of Patients 2, 3, and 4 at autopsy, and in some of the hepatocytes and bile duct epithelial cells of the liver removed at the time of hepatic transplantation from Patient 2.

Viral studies. CMV was isolated from the liver homografts, and

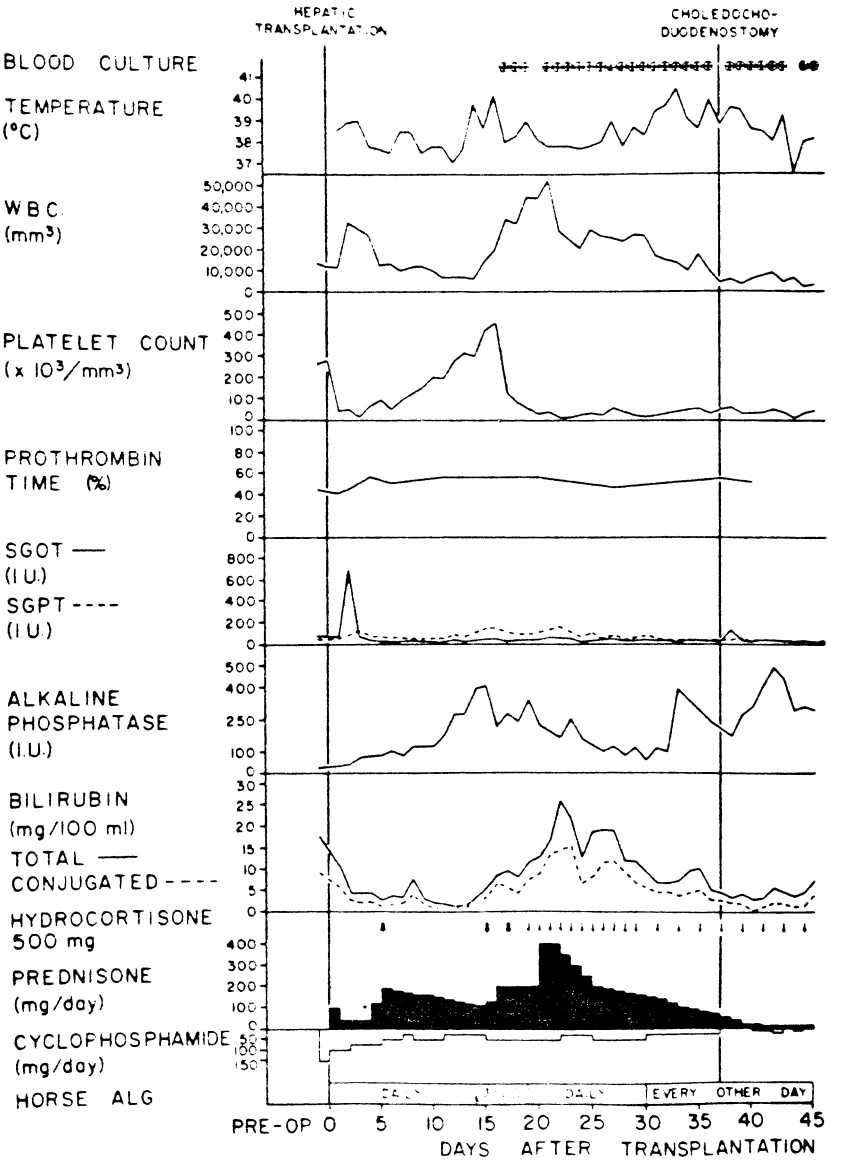

Fig. 2.— The course of a 16-year-old recipient of a hepatic homograft (Patient 3). Although liver function was satisfactory initially, hyperbilirubinemia developed 15 days after operation, but receded slightly with intensification of immunosuppression. Systemic sepsis prompted re-exploration, at which time a cholangiogram (Fig. 3, C) was obtained and biliary diversion was converted to choledochoduodenostomy. However, the child died of uncontrolled sepsis 46 days after transplantaion.

several other tissues obtained at autopsy from Patients 2, 3, and 4. In Patient 2, the virus was also cultured from the patient's own liver which had been removed two months previously. Typical intranuclear and intracytoplasmic virus particles were found on electron microscopic study of the cytomegalic cells from some of these tissues.

DISCUSSION

Extrahepatic biliary obstruction after orthotopic liver transplantation has been noted before, but it usually resulted from technical errors or from the recurrence of malignancy.[3] Delayed cystic duct obstruction of the kind herein reported has been decsribed[4] in only one patient, the same child (OT 12) as Patient 1. Three more examples have now been encountered, giving an incidence of 10 percent in our series for this lethal complication.

In each of the four instances, bile drainage seemed satisfactory at the time of operation, and, furthermore, it was free enough to permit clearing of pre-existing hyperbilirubinemia during the initial convalescence. Then, 3 to 22 days postoperatively, jaundice deepened. Rejection was diagnosed and treated with increased doses of prednisone.

The diagnosis of rejection seemed supported in three of the four cases by the partial reversal of the secondary hyperbilirubinemia. Nevertheless, even these three patients continued to have low-grade jaundice. All four recipients became febrile and developed bacteremia and leukocytosis. The clinical picture of life-threatening cholangitis was now complete. All of the recipients eventually died from intrahepatic and systemic sepsis; two patients died in spite of reoperation and secondary construction of choledochoduodenostomy. In the latter two patients, the provision of adequate biliary drainage was too late since multiple foci of infection within the liver were already well established. At either autopsy or reoperation, the

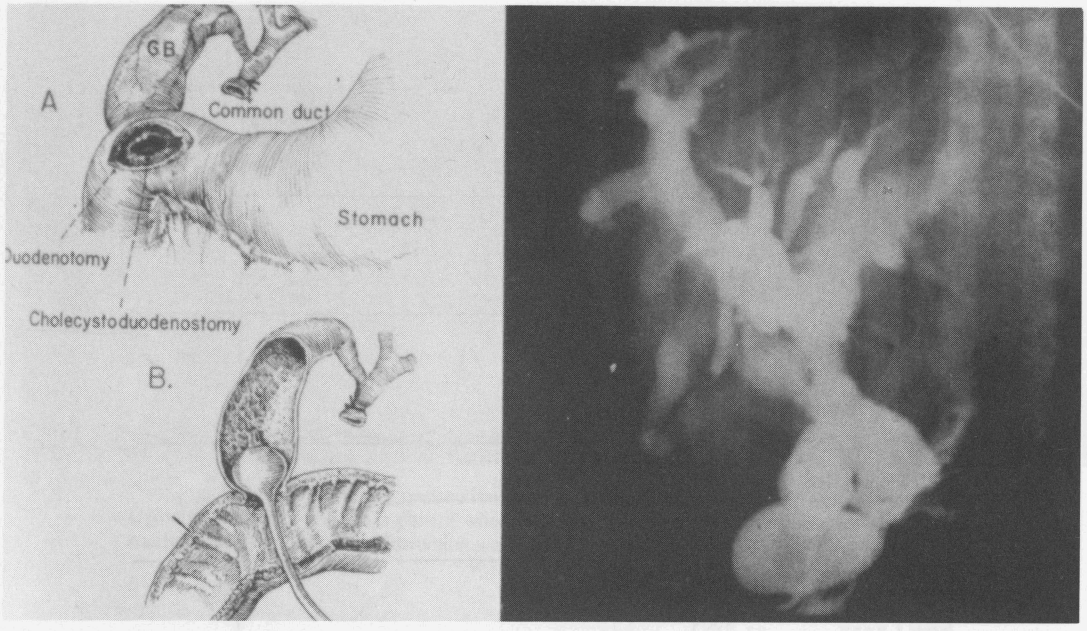

Fig. 3.— Cholangiography of hepatic homograft. A and B, Technique of dye injection through a duodenotomy and the anastomosis. C, Obstructed duct system in Patient 3.

Table II. *Pathological features of four obstructed hepatic homografts**

Case No.	Liver						Gallbladder and cystic duct	
	Hepatic duct dilatation	*Intra-hepatic abscesses*	*Cholan-gitis*	*Cytomeg-aloviral lesions*	*Re-jection*	*Vascular supply*	*Chole-cystitis*	*Cytomeg-aloviral lesions*
1	++++	–	±	–	±	Right hepatic artery thrombosed	+++	–
2	++++	+	±	+	–	Normal	±†	++†
3	++++	+	+++	±	–	Normal	+	+†
4	++++	+	++	++	–	Normal	++++	+++

*Grading System: –, none; ±, minimal; +, mild; ++, moderate; +++, advanced; and ++++, severe.

†Specimens obtained at reoperation.

intrahepatic ducts and common ducts of the four homografts had become remarkably dilated and filled with bile and a soft chalklike debris. There was collapse of the cystic duct and gallbladder.

The etiology of the biliary obstruction probably involved other factors than simple positional distortion at or near the junction of the common and cystic ducts, since the mechanical arrangement in each case was initially satisfactory for bile drainage. It is possible that acute rejection played a precipitating role with swelling and consequent occlusion of the cystic duct lumen particularly very early in convalescence. With partial reversal of the process, as seemed to have been achieved in three of the four patients by intensified immunosuppression, bile flow through the cholecystoduodenostomy could have resumed, although suboptimally. However, in the specimens examined at reoperation or autopsy, there was no real evidence to indict present or past rejection in etiology of the delayed obstruction.

In contrast, the development of cholecystitis or viral inclusion disease in the homograft gallbladder could have been a factor particularly after the first few weeks. One or both lesions were present in the gallbladders of each of the four cases. In one patient, there was evidence that the cytomegalic disease was established before hepatic transplantation. The viral infection might have been particularly significant because, recently, it has been shown that infection with a Papovavirus of the epithelial cells lining a homograft ureter can produce obstruction.[2] The obstruction in cases of either renal or hepatic homografts could presumably be secondary to the kind of ulceration with or without healing that CMV can produce in the urinary or gastrointestinal tracts[3] or by the shedding of swollen cells containing intranuclear viral inclusions which could contribute to the obstructing debris. Whatever the exact mechanism, the small cystic duct would be preferentially susceptible to the mechanical consequences of all events.

One way to minimize biliary obstruction of the kind documented in this report would be to abandon cholecystoduodenostomy as the primary means of biliary reconstruction and, instead, to employ one of the standard techniques of common duct anastomosis combined with cholecystectomy. Unfortunately, the methods employing the common duct have also carried a high risk after liver transplantation, mainly because of anastomotic leak.[1,4] Moreover, a secondary operation to correct such a leak provides limited options, since the prior removal of the gallbladder eliminates the possibility of delayed cholecystenterostomy. Finally, the use of the common duct for anastomosis would not preclude obstruction secondary to infestation by CMV. If this virus proved to have an important causal relationship to duct obstruction, a more sensible approach would be to consider antiviral treatment as, for example, with cytosine arabinoside. Under immunosuppression, it is known that a very high percentage of whole organ recipients are chronic carriers of CMV although until recently there has been little evidence that this virus causes important sequelae.

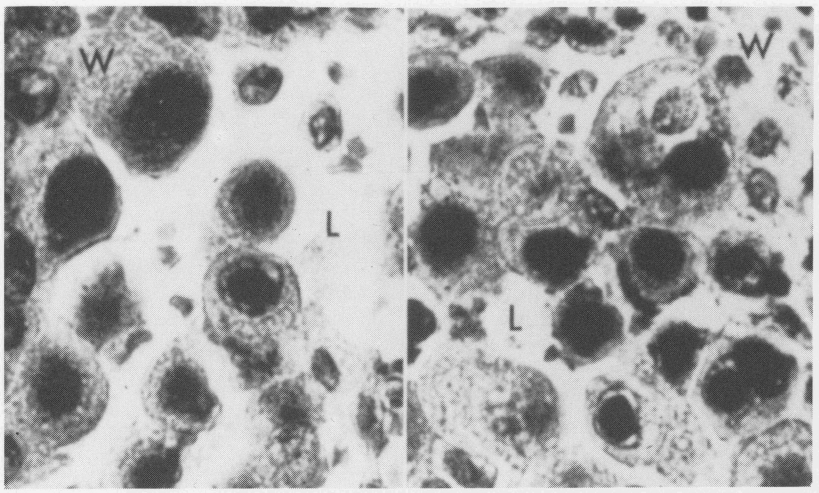

Fig. 4.— Obstructed cystic ducts of hepatic allografts in Patients 2 (A) and 4 (B). The wall (W) of each duct is lined by swollen epithelial cells which contain cytomegaloviral inclusions. Some similar cells lie free in the lumen (L) of each duct. (Hematoxylin and eosin. X900.)

It is our present view that cholecystoduodenostomy is still the safest way to reconstruct the biliary system initially after liver transplantation, since the procedure can be done without internal stents or drainage and because it burns no anatomic bridges if reoperation becomes necessary. However, with cholecystoduodenostomy, there must be a firm commitment to perform a re-exploratory operation for very specific indications. These indications consist essentially of the clinical features of cholangitis, including evidence of obstruction, a septic course, and, above all, persistent bacteremia. The first two of these findings can be seen with simple rejection, but continuous bacteremia should raise the highest suspicion of a surgically correctible complication. At operation, the cholecystoduodenostomy could then be converted to choledochoenterostomy if warranted by the findings of transduodenal cholangiography. In two of the presently reported cases, the significance of the bacteremia was not appreciated until autopsy, and in the other two corrective surgery was delayed so long that all hope of succes was lost.

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This article was presented at a tribute to David Hume in Richmond, Virginia, almost one year after Hume's death in an airplane accident. New information was given including: recognition that biliary tract complications were occurring from inappropriate techniques of biliary reconstruction; a description of the resistance of the liver to hyperacute rejection from pre-formed antibody states; a description of a third chimpanzee-to-human heterograft; and a discussion of the effect of native liver disease on the outcome of transplantation. All of these lines of inquiry were followed in the succeeding years.

Progress in and deterrents to orthotopic liver transplantation, with special reference to survival, resistance to hyperacute rejection, and biliary duct reconstruction

Transplantation Proceedings, 6: 129-39, 1974

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Before I begin, I want to add my own personal reminiscence. I knew Dave Hume for almost 14 years, slightly for the first 4 and well for the last 10. I first talked to him at an elevator entrance at the Greenbrier Hotel in West Virginia, in April, 1959, and for the last time in April, 1973, in the lower lobby of the Century Plaza Hotel in Los Angeles. In May, 1973, I was in the railroad station in Albuquerque, New Mexico, when I learned from my grief-stricken youngest son that Dave was dead. It is strange how the exact details of these and some other memories in between, of the time I spent with Dave Hume, stand out with the same clarity as what I was doing when I learned of the bombing of Pearl Harbor, the assassination of John Kennedy, but very few other things. The most eloquent tribute to Dave Hume I have heard was the briefest, coming from a non-medical friend who told me sadly," He really *was* a dynamite guy!"

There is almost no aspect of clinical organ transplantation into which Dave Hume did not breathe life. Liver transplantation was no exception. It will surprise no one that his contributions were important and concisely stated, although never published. He gave his observations to one of us (T.E.S.) as personal communications throughout the years and granted permission for their use in a book published 5 years ago.[1] They should be listed briefly.

Hume performed one of the earliest auxiliary hepatic transplantations, to the splenic fossa of a recipient whose abdomen could not accommodate the extra organ plus the host liver. Undaunted, he proceeded to remove the total native liver. In another trial, this time with orthotopic transplantation, Hume described hyperacute rejection, which if it was a valid diagnosis was the first only documented example of this complication destroying a liver homograft. Finally, one of Hume's recipients who had a hepatoma plus cirrhosis lived for about a year postoperatively after liver replacement, eventually dying with widespread metastases similar to those we have recorded after hepatic transplantation for the indication of malignancy.[1]

There is no point in saying more about these experiences of Hume, since, important as they were, they were really peripheral to his main interests. Instead, I would like to discuss three aspects of orthotopic liver transplantation that might introduce either new data or new ideas. These concern our survival statistics, hyperacute rejection in livers, and the problem of biliary duct reconstruction.

Survival Statistics

According to the April 1974 report on liver transplantation being prepared by Dr. Carl G. Groth for the American College of Surgeons Registry, about 200 patients have had liver replacement.[2] Since 1963 we have contributed 82 to this total, at a rate since 1967 ranging from 6 to 13 per year (Table 1). We have had 18 and 9 recipients, respectively, who have lived for more than 1 and 2 years. Thirteen recipients are still alive from 2 weeks to almost 5 years postoperatively. The 4 longest survivors are 4 years, 10

Table 1. Cases of Orthotopic Liver Transplantation Treated in Denver

Years	Number	Lived 1 year	Lived 2 years	Alive Now
1963-1966	6	0	0	0
1967	6	1	0	0
1968	12	5	2	0
1969	6	2	1	1
1970	10	2	1	1
1971	11	2	2	2
1972	11	5	3	3
1973	13	1	0	3
1974 (to April 1)	7	0	0	3
	82	18	9	13

Table 2. Present Status of 18 One-Year Survivors after Orthotopic Liver Transplantation. Eight Are Still Alive from 14 to 58 Months. The Other 10 Eventually Died from the Causes Listed Below

OT Number	Time of Death (months)	Cause of Death
15	12	Recurrent cancer
29	12	Serum hepatitus and liver failure
8	13	Recurrent cancer
58	13½	?Chronic rejection ?Recurrent hepatitus
16	13½	Rejection and liver failure
14	14	Recurrent cancer
54	19	Multiple liver abscesses necessitating retransplantation
36	20	Systemic *Nocardia* infection and chronic aggressive hepatitis
13	30	Rejection and liver failure following retransplantation
19	41	*Hemophilus* septicemia and secondary liver and renal failure

months; 4 years, 4 months; 3 years, 10 months; and 3 years, 2 months.

There have been 10 late deaths, from 12 to 41 months postoperatively, and for the reasons listed in Table 2. The latest mortality was at 3 years, 5 months, following a bout of *Hemophilus* septicemia (OT 19). The homograft arteries contained the same kind of occlusive lesions that have been seen in renal transplants.[3]

The causes for the high acute failure rate have been discussed elsewhere.[1] The single most important factor has been a multiplicity of technical misadventures of which complications of biliary duct reconstruction lead the list (see next section). Poor control of rejection and systemic infection are the next leading causes of death.

The Strategy of Bile Duct Reconstruction

As was just mentioned, the Achilles' heel of liver transplantation has been biliary duct reconstruction. The different techniques we have used to restore bile drainage include choledochocholedochostomy with or without a T tube (not applicable with biliary atresia), cholecystoduodenostomy after ligation of the graft common duct, and choledochoduodenostomy. Because of continuing dissatisfaction with all of the aforementioned techniques of duct recontruction, we have recently embarked on a trial of Roux-en-Y cholecystojejunostomy (see later under Possible Solutions). The lethal complications with most or all of these procedures were of two general kinds, one obvious and proved and the other subtle and still speculative.

Table 3. Kind of Primary Bile Duct Reconstruction Used in 82 Consecutive Cases of Orthotopic Liver Transplantation

	Cholecystoduodenostomy	Choledochocholedochostomy	Roux-en-Y Cholecystojejunostomy	Choledochoduodenostomy	Cholecysto-Loop Jejunostomy	Total
Number	59	9	8	4	2	82
Obstruction	15	0	2	0	0	17*
Fistula	2	5	0	1	0	8*

*In these 25 cases, reoperations were performed in 13 patients with attempt at duct reconstruction. A satisfactory recovery followed in only 4 of the recipients. Two later died after 6 and 13½ months post-transplantation survival. The other are alive after 3 months and 2 years, respectively. Both survivors now have the final biliary duct reconstruction shown in g. 2C.

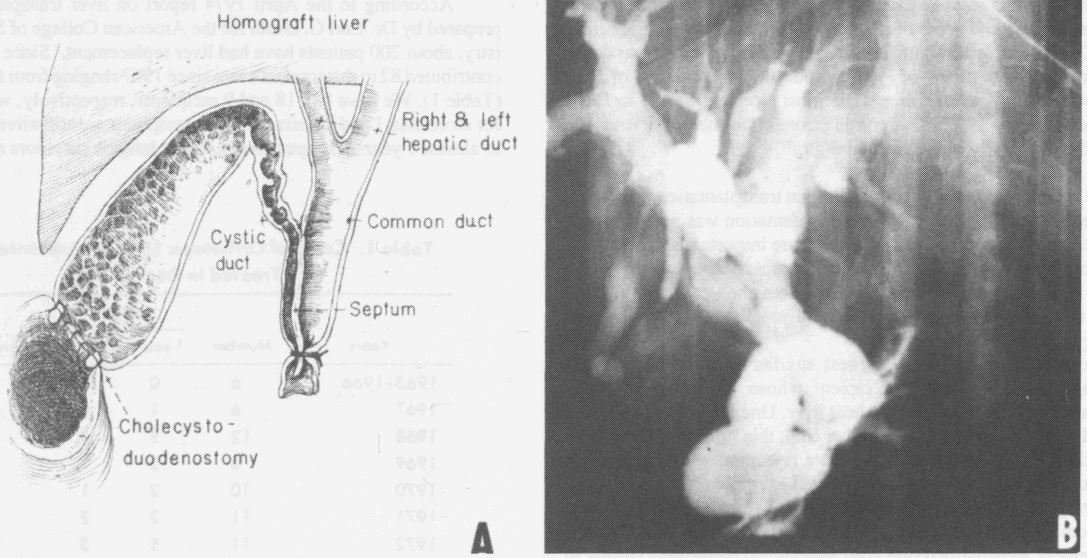

Fig. 1.— Two kinds of biliary duct obstruction after cholecystoduodenostomy. (A) The anatomic basis for a technical error that cost the life of 3 patients. Distal ligation of the double-barreled extrahepatic duct system resulted in total biliary obstruction. This recurrent accident has caused us to perform cholangiography on all liver homografts before transplantation. (B) The kind of biliary obstruction caused by stenosis of the cystic duct. Martineau reported that cytomegalovirus infection of the duct could be responsible for this development.[4]

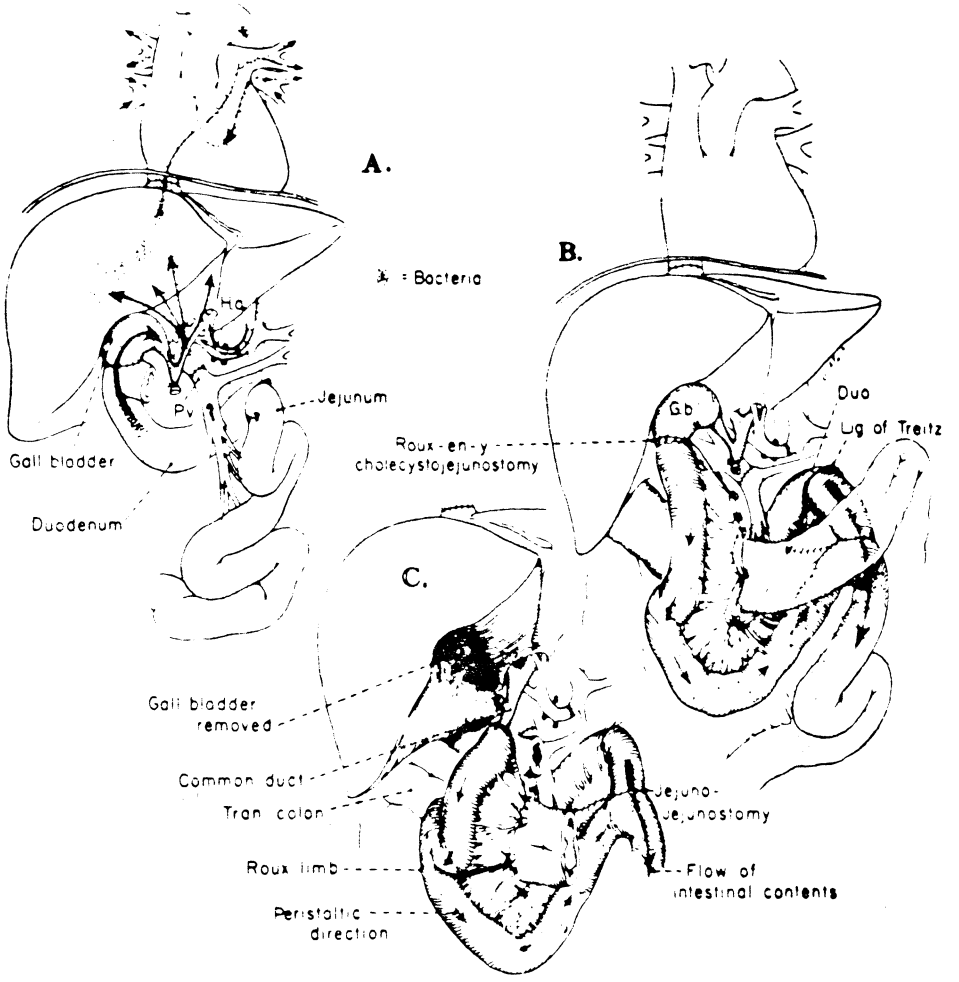

Fig. 2.— Schematic representation of the bacterial contamination or lack thereof in three different kinds of biliary reconstruction. (A) Cholecystoduodenostomy. This extremely simple operation probably carries the greatest risk of graft infection. (B) Roux-en-Y cholcystojejunostomy. This operation protects from hepatic sepsis by placing the new liver outside the main gastrointestinal stream. The isoperistaltic limb is made at least 18 in. long. (C) Roux-en-Y choledochojejunostomy. The end-to-end duct-to-bowel anastomosis is simple if the duct is dilated, as would be the case if a conversion became necessary from B to C.

Statement of the Problem

Mechanical problems. The obvious biliary duct problems have been obstruction and biliary fistula from anastomotic leaks. In our 82 cases of orthotopic liver transplantation the initial biliary reconstruction was eventually shown to be unsatisfactory, leading either to death or early reoperation in 25 cases (Table 3), for the staggering incidence of 30%; the true frequency was undoubtedly even higher, since many patients died so early postoperatively that an incipient duct problem would not yet have been manifested. In 13 of the 25 recipients an effort was made at secondary repair. Even in these 13 reoperated cases the biliary duct problem was an important contributory or the main cause of death in at least 9.

None of the commonly used methods of biliary duct reconstruction was trouble-free (Table 3). With cholecystoduodenostomy, fistulae were uncommon, but obstruction occurred in 25% of cases. The obstructions ranged from accidental acute ligation of the cystic duct before performing cholecystoduodenostomy (Fig. 1A) to delayed obstruction (Fig. 1B) of the cystic duct in some cases, apparently due to cytomegalovirus (CMV) infection weeks or months postoperatively.[3] Most commonly, no obvious etiologic cause was evident, accounting for the partial cystic duct obstruction. With choledochocholedochostomy or choledochoduodenostomy the leading complication was biliary fistula formation.

There were two obstructions with Roux-en-Y cholecystojejunostomy. In one, the kind of cystic duct ligation shown in Fig. 1A had not been recognized and was not diagnosed until autopsy. In the other case, there was partial obstruction (Fig. 3B) of the cystic duct necessitating conversion of the ultimate hookup shown in Fig. 2C).

Special bacteriologic complications. With the well-defined technical complications cited above, clinical evidence of cholangitis (including bacteremia) is easily understandable and is often accompanied by histopathologic findings of cholangitis. In addition, a subtle and as yet hypothetical complication may occur in spite of an apparently satisfactory biliary duct reconstruction. It has been reported by us that systemic infection and even asymptomatic bacteremia are common problems in liver recipients.[1] For years there has been strong justification to believe that the transplanted liver itself was the portal of entry by which microorganisms of all kinds gained access to the bloodstream. The variety of bacteria that were cultured from peripheral veins of patients, both early and many months after operation, was strikingly similar to that found in dogs and pigs subjected to liver injury or hepatic transplantation.[5] In the human liver recipients with bacteremia the failure to find any other focus of infection necessitated indictment of the homograft (as a site of entry) by the process of exclusion. The two routes of entry could be the portal vein or the duct system, but the former possibility seems less and less important.

The exposed relation of the duct system of the orthotopic liver to gastrointestinal flora is probably the first step in bacterial "leak" through

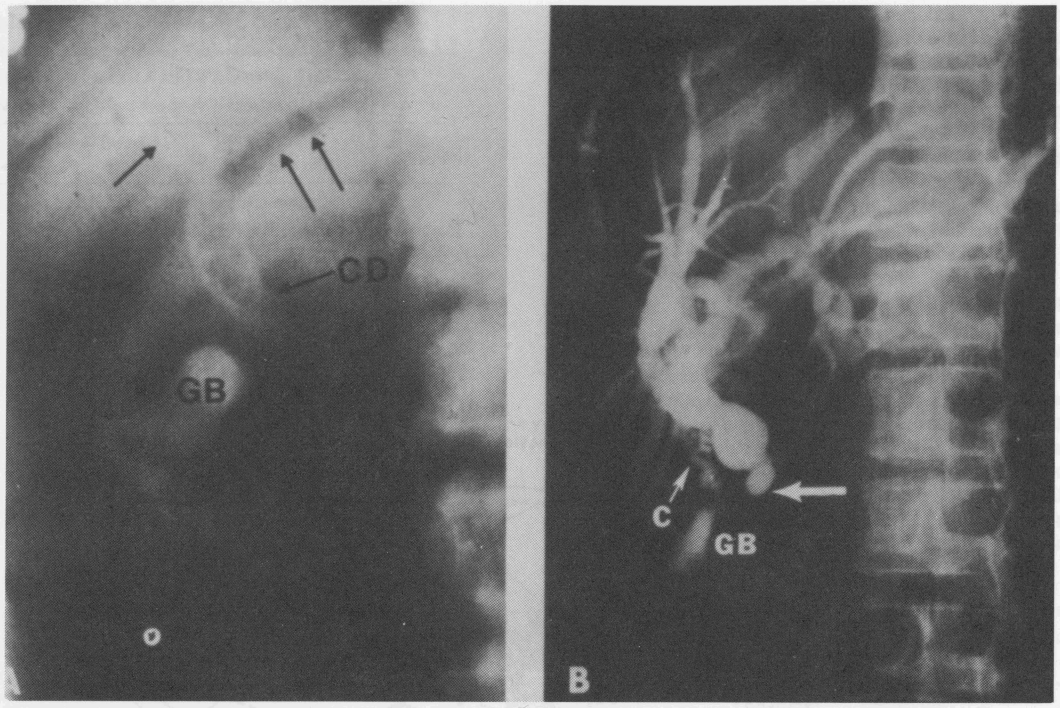

Fig. 3.— Post-transplantation cholangiographic studies. (A) Intravenous cholangiogram in a 47-year-old recipient of a hepatic homograft, the biliary drainage for which was with Roux-en-Y cholecystojejunostomy (Fig. 2B). The patient's liver function studies were normal at the time of the examination. However, the findings of a very slightly dilated common duct and air in the biliary system (arrows) are suspicious for low-grade obstruction. (B) A percutaneous transhepatic cholangiogram performed 4 weeks post-transplantation because of persistent elevations of the serum bilirubin (8-10 mg/100 ml). At the time of transplantation, biliary drainage had been established with a Roux-en-Y cholecystojejunostomy (Fig. 2B). After obtaining this study, the patient was re-explored, the gallbladder removed, and the Roux limb anastomosed to the dilated common duct (large arrow), as shown in Fig. 2C. The patient's jaundice rapidly cleared, and he now has normal liver function 3 months post-transplantation. GB, gallbladder; CD, common bile duct; C, cystic duct.

the homograft, which may well be bacteriologically porous without the presence of histopathologically significant cholangitis. This situation after cholecystoduodenostomy is depicted in Fig. 2A. If bacteria enter the circulation through the duct system of hepatic homografts, the logical solution would be to carry out liver transplantation as far removed from the mainstream of the gastrointestinal tract as is possible, as has been illustrated in Figs. 2B and 2C.

Practical Solutions

The five guiding principles we are now attempting to follow are: (1) avoidance of stents or drains; (2) preservation of maximum extrahepatic biliary duct tissue; (3) intensification of diagnostic efforts to differentiate between duct obstruction and rejection, including performance of cholangiography in all homografts prior to transplantation; (4) early reoperation for suspicion of obstruction; (5) placement of the liver in a relatively bacteria-free relation to the mainstream gastrointestinal continuity. None of the presently available operations completely meets all of these objectives, so that considerable individualization of care is necessary.

A Roux-en-Y cholecystojejunostomy (Fig. 2B), our present procedure of choice, permits all the above listed objectives to be partly met. If postoperative biliary obstruction later develops, the Roux limb can be detached, the gallbladder removed, and an anastomosis performed to the now dilated common duct (Fig. 2C).

The most important objection to this approach is that a Roux-en-Y cholecystojejunostomy can be an extremely difficult added procedure at the end of a long and arduous liver transplantation. The typical adult liver recipient is dying of hepatic failure and has massive collaterals in the small-bowel mesentery. In addition, the mesentery is usually thickened and waterlogged with edema fluid. Construction of a Roux-en-Y isolated limb under these conditions may require 3 to 6 additional hours of operating time in a patient who has already sustained thousands of milliliters of blood loss. Under these adverse conditions, it may be the better part of valor to perform a simple cholecystoduodenostomy with the objective of returning later.

If at the time of transplantation the gallbladder were found to be defective, we would then make a selection between choledochocholedochostomy with T-tube stenting, and a Roux-en-Y choledochojejunostomy.

No matter what the initial procedure, an intense suspicion about the cause for postoperative jaundice is a necessary condition of postoperative management. The simplest precaution is to perform routine intravenous cholangiography in the early postoperative period (Fig. 3A). In almost all of our patients who develop jaundice, transhepatic cholangiography (Fig. 3B) and percutaneous needle biopsy are now performed. Cholangiography has been greatly expedited by our use of the Chiba needle introduced in Japan[6,7] and now being used in several American centers. These thin-walled small-caliber needles have great flexibility that permits the diagnostic studies to be done with an improvement in safety (Fig. 3B).

It is not yet established that these changes in policy will improve the results after liver transplantation. Our approach is fundamentally different from that proposed by Calne, who believes that duct-to-duct reconstruction over a T-tube and preservation of the sphincter of Oddi will be the better solution.[8] The fact that different methods are being tried to solve a generally recognized set of problems should be of advantage in evolving solutions that can eventually be agreed upon.

Hyperacute Rejection

The pathophysiology of hyperacute rejection has been well worked out in recent years. Fixation of antibody to the transplant is apparently the initiating event, as was first noted in kidney homografts after breaches of red-blood-type barriers.[9] In later years the predominant cause of hyperacute rejection has been the presence in the recipient of antigraft cytotoxic

Table 4. Three Cases of Orthotopic Transplantation of ABO-incompatible Livers

OT Number	Age (years)	Diagnosis	ABO Types Donor → Recipient	Preoperative Isoagglutinin Titer	Survival (days)	Cause of Death	Pathologic Changes in Liver
59	11/12	Biliary atresia	AB → A	1:4 (anti-B)	173	Septicemia (from liver?)	(Arterial and arteriolar narrowing (past rejection)
60	46	Primary biliary cirrhosis	AB → A	1:32 (anti-B)	61	Septicemia (from liver?)	Mild cytomegaloviral infection
						Pulmonary emboli	No rejection
61	42	Postnecrotic cirrhosis	A → O	1:512 (anti-A)	41	Disseminated herpes and cytomegalovirus	Cytomegalovirus infection
						Pulmonary emboli	No rejection
						Brain infarction	

antibodies, as was first described by Terasaki[10] and confirmed by Kissmeyer-Nielsen[11] and others.[12-14]

In experimental animals of widely disparate relationship, an experiment of nature with hyperacute rejection may be constructed, as for example in transplanting organs from pigs to dogs.[15] The serum of dogs contains heterospecific antiporcine cytotoxic antibodies.

With either homografts or heterografts transplanted to recipients that possess preformed antigraft antibodies, the actual destruction of the homograft or heterograft is a complex process in which formed blood elements and clotting factors are entrapped by the graft.[13-15] The resulting occlusion of the major vessels causes ischemic necrosis and a characteristic purple or mottled appearance.

It is probable that the kidneys, because of the special filtering properties of the renal microvasculature, are unusually prone to the irreversible consequences of hyperacute rejection. In contrast, the liver may be unusually resistant, as the ability of pig livers to perform rudimentary functions for a number of hours while being perfused with human blood might have predicted.[16] Even in the difficult pig-to-dog heterograft model in which kidneys are grossly rejected in a few seconds the liver often does not suffer this fate for more than an hour.[15]

The resistance of the liver to hyperacute rejection may prove to be sufficiently great to permit transplantation under conditions that would be categorically unacceptable for kidneys. If so, an important stricture on the practicality of the procedure will be eased. Patients dying of liver disease usually cannot wait for an ideal homograft. If transplantation could be conducted in spite of preformed antibody states, patients deprived in the past of a trial at treatment would no longer be arbitrarily excluded. In this connection, our previously unreported experience is of potential interest.

Table 5. Serial Antigraft Isoagglutinin Titers in the Three Recipients of ABO-incompatible Livers Described in Table 4

Post-transplantation Day	OT 59 (Anti-B)	OT 60 (Anti-B)	OT 61 (Anti-A)
0	1:4	1:32	1:512
1	1:4	1:16	
3	1:1	1:4	1:64
5	1:1	1:2	1:64
7	1:1	1:8	1:2048
9	1:4	1:64	1:8192
11	1:4	1:64	1:8192
13	1:4	1:32	1:4096
15	1:4	1:16	1:2048
17	—	1:8	1:1024
19	1:2	1:4	1:1024
21	1:1	1:4	1:512
28	1:1	1:2	1:256
35	1:2	1:2	1:128
42	1:2	1:2	
49	1:2	1:1	
56	1:2	1:8	
63	1:2		
70	1:2		
77	1:8		
84	1:4		

ABO incompatibility. In 1972, 3 patients with ABO-mismatched livers were transplanted to recipients whose conditions were considered sufficiently grave that they could not wait (Table 4). Hyperacute rejection did not occur, and no obvious adverse consequences were seen. The titers of antigraft isoagglutinins were highly variable, and at least in one case reached prodigious levels (Table 5). Eventually the three patients all died, but the pathologic findings were remarkably minor. The homograft of 1 of the patients (OT 60) became partially obstructed by the mechanism of cystic duct stenosis shown in Fig. 2B; following biliary reconstruction, the recipient died of pulmonary sepsis. The other 2 patients had almost no abnormalities in their livers when they died of infectious complications.

Cytotoxic antibodies. This ability of the liver to remain healthy under conditions that would be predictably harmful to most kidneys is a noteworthy feature that has been seen in other preformed antibody situations. During the last 2 years, 3 patients with antidonor cytotoxic antibodies have been given livers. In all 3 cases cytotoxins were also present against most of the donors of an indifferent lymphocyte screening panel. Thus the prospects of finding a liver without a positive cytotoxic antibody cross-match were considered nil. As a consequence, a decision was made to proceed despite the potentially adverse prognostic implications.

None of the 3 patients developed hyperacute rejection, although they all eventually died from 3-1/2 weeks to 13-1/2 months later (Table 6), in 2 cases with relatively good livers. In OT 71, the homograft seemed to have been severely damaged by ischemia, as well as cellular rejection, although its poor initial function could have been a manifestation of acute antibody-mediated injury. After 10 days the organ was removed and replaced by a chimpanzee heterograft, against which the recipient cytotoxins also reacted. The chimpanzee liver functioned for most of the 14 subsequent days of the patient's life. Upon pathologic examination the initial homograft had many focal areas of necrosis compatible with the diagnosis of ischemic injury. In contrast, the heterograft was well preserved. Centrilobular cholestasis was a prominent feature. Otherwise, there was little evidence of rejection. This was our third trial of chimpanzee-to-man heterotransplantation, the other two having been previously reported.[1,17]

It goes without saying that preformed antibody states should be avoided if at all possible. However, the experience cited both with the ABO red cell and cytotoxic antibodies makes it possible that this kind of positive cross-match is not an absolute but only a *relative* contraindication for liver transplantation.

Summary

An account of 82 consecutive orthotopic liver transplantations carried out in Colorado is given. Eighteen patients have lived for 1 year postoperatively, and the longest survival is almost 5 years.

Table 6. Three Cases of Orthotopic Hepatic Transplantation in which the Recipients Had Antidonor Cytotoxic Antibodies

OT Number	Age (years)	Diagnosis	Preoperative Cytotoxicity Titer	Survival	Cause of Death	Pathologic Changes in Liver
58	34	Chronic aggressive hepatitis	1:2	407 days	Stopped immunosuppression Hepatic insufficiency	Resolution of previous obstructive changes at 8½-month biopsy (no autopsy)
63	49	Primary biliary cirrhosis	1:64	26 days	Gastrointestinal hemorrhage	Normal liver
71	1 11/12	Biliary atresia	1:16 (homograft)	(Removal of the graft at 10 days)		Acute rejection, cellular and humoral
			1:16 (heterograft)	14 days after retransplantation	Pulmonary edema, bronchial hemorrhage	No evidence of cellular rejection. Centrilobular cholestasis.

Much of the high failure rate is attributable to the technical difficulty of the operation and especially to complications of the biliary duct reconstruction. A strategy for biliary duct reconstruction is advanced that is designed to place the liver as far outside the mainstream gastrointestinal tract as possible, to avoid unnecessary sacrifice of biliary duct tissue and to facilitate reoperation at the slightest sign of a technical complication.

An experience is cited in 6 patients who received 6 homografts and 1 chimpanzee heterograft in which livers were transplanted against preferred anti-red-cell isoagglutinins or leukocyte cytotoxins. Hyperacute rejection did not occur, nor was there convincing evidence of antibody-mediated rejection in any case. The conclusion is that the liver may be more resistant to hyperacute rejection than is the kidney.

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Discussion

Dr. Calne: Tom, I would like to congratulate you on the tremendous amount of work that you presented here. I think that the conclusions that you have come to about biliary drainage will be very interesting to compare with the results you get with the long Roux loop and what we get with the T tube. One potential advantage of the T tube is that you can do a cholangiogram every day if you want to, and you can be sure at any rate whether there is a major blockage. I think there is one aspect of liver transplant that perhaps deserves a little bit of emphasis, and that is the diagnosis of rejection. This can be exceedingly difficult even by biopsy. The changes that can occur in the liver are relatively limited, and to give a dogmatic opinion that the changes are of rejection, infection, drug toxicity, or ischemia can be difficult. At our last liver transplantation we had a rather interesting experience, which you probably have observed also, of a long ischemic period. The liver was removed some 60 miles away and brought to our hospital, and the behavior of it at operation looked fine. The patient became progressively more and more jaundiced and the bilirubin went up to 40. There was no evidence of rejection on the migration of white cells test, which we think is quite a useful test. The T-tube cholangiogram showed that the ducts were quite normal. We didn't increase immunosuppression. We just sat tight and the bilirubin came down spontaneously to normal, and the patient is out of the hospital now. I think that previously we would have panicked and given huge doses of immunosuppression and probably killed the patient with infection. It emphasizes some of the discussion that was held this morning about not giving extensive amounts of immunosuppressive agents. The other patient we have living now more than a year is not on any steroids at all, and just on 120 mg. of Imuran a day. I think that this experience in patients with livers on lower doses of immunosuppression than kidneys goes along very well with the observations that you described of crossing the ABO barriers and crossing cross-match barriers, and fits in with our own thoughts very closely, both experimentally and clinically; if needed we can use any kind of donor on ABO grounds for liver transplantation it would make the procurement of donors and the actual treatment of patients very much easier and be a great important logistic advance.

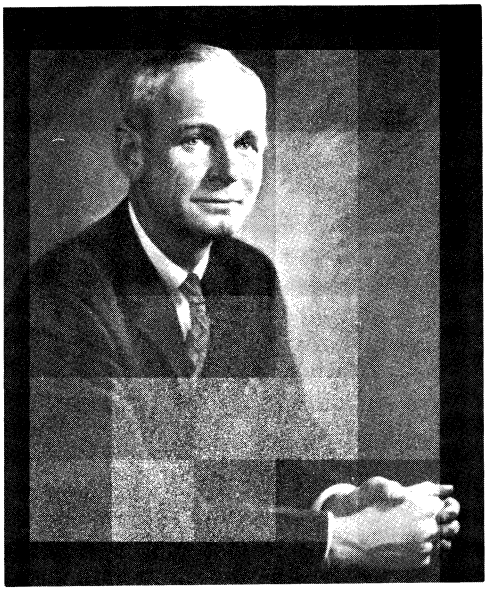
Dr. A. G. Birtch: (Springfield) I, like Dr. Calne, would like to compliment Tom on a beautiful presentation. I would have just two comments. One, actually the first liver transplant that we did at the Brigham was indeed ABO-incompatible. It was B to A, and we did indeed find anti-B antibody; and that liver as you recall was one that had suffered significant ischemic damage. We were never able to put together whether the findings at day 10 or 11 when the patient succumbed were due to the ischemic injury, which we assumed, or had anything to do with the anti-B titer that was present. I still don't know. If I understand your 3 patients correctly, none of them were long-term survivors. I wondered if there was anything in the long-term course of those patients that made you think that crossing that barrier had anything to do with their not becoming long-term survivors. Second, I would just like to reminisce a bit myself. As you remember, Tom, in Dr. Moore's original technique he used the Roux-en-Y in the dog for many years, feeling at that time that it had to be better than the duodenostomy. One of the things that we did later in trying to trim the operation down to size was to abandon that technique and go to your technique of putting the gallbladder into the duodenum. I have learned this lesson many times personally; I don't know whether it is the answer eventually, but I've found that when I change something that Dr. Moore has done, I usually come back to regret it in the end.

Dr. Starzl: I agree with you about confronting Dr. Moore's opinion, and I try assiduously to avoid that. As to the Boston case, the blood type direction was B to A, a detail I know because you and Dr. Moore made the facts available to me. I have had the occasion recently to go back and look at what you gave me when it was fresh in your mind. That liver developed multiple intrahepatic abscesses as the most important postmortem finding, something that I think would not necessarily be related to the confrontation of the blood type. For a long time I thought it would be unlikely that we should do such red-blood-type breaches anymore, but I obviously changed my mind to some extent. The time of death of one of those ABO failures was about 40 days. That was the patient who had a hepato-renal syndrome that reversed, but the brain lesion didn't go away. We were left with a

vegetable whose systemic support was eventually discontinued. The second case was a child with biliary atresia who had a perfect result and who died some 7 months later from the kind of complication that apparently killed Roy Calne's long-surviving patient last week, and which killed one of our longest survivors, namely septicemia. The third combination was an AB to A death that occurred about 2 months or so postoperatively, and followed an attempt to correct a biliary duct obstruction. I think these cases had no evidence of antibody damage. As to the 3 with cytotoxins, only case 3 might have had antibody damage. In that liver there are findings that could be explained by immunologic or ischemic damage. I am fascinated by Calne's case with the high-titer cytotoxins, and although one might become incontinent at the moment of revascularization I think I might try confronting a cytotoxic cross-match again if I really had to do it.

Dr. Wolf: Just two more general questions. One, what is your current feeling on transplanting for malignant disease? And secondly, how about your current immunosuppression for these patients?

Dr. Starzl: I think the patient with malignant disease is a bad risk, but it's obviously a matter of judgment as a clinician when you are confronted with a case; in spite of all kinds of resolutions about not wanting to do more tumor cases you end up treating the patient. Not long ago I had such a patient with a duct-cell carcinoma. I was skiing in Aspen at about the time the patient arrived, and when a donor became available I did him because there was a donor and because it obviously was his only chance. As a matter of fact, I have thought for a long time that this kind of neoplasm might be a good indication because the tumor itself is so small. At the time of the liver replacement in the case I just mentioned, I never had a positive tissue diagnosis until the case was all finished. Even then the pathologist had to go on a detective hunt to find the tumor. It was very small and partly necrotic. The cases that we have had difficulty with have been the hepatomas that were already big. Even then we know that it is possible to effect a cure. Roy's case has demonstrated this. We have a child at 4 years, 4 months who obviously represents a cure of a hepatoma, and there are some others around. A few. So I think that under properly defined circumstances it would be reasonable to do a few of these, and so I don't have a closed mind about it.



William R. Waddell

William R. Waddell was Chairman of the Department of Surgery at the University of Colorado, and he witnessed the many complications from biliary tract reconstruction in liver recipients. He proposed a technique that subsequently was used routinely by Calne in Cambridge. Ironically, it was not tried by the Colorado-Pittsburgh surgeons until much later. In 1972, Waddell left the chairmanship at the University of Colorado, and, in 1985, he retired from university life to a busy private practice in Silver City, New Mexico.

Today Grover is Professor of Surgery at the University of Texas Health Sciences Center at San Antonio and Chief of the Cardiothoracic Service at the Audie Murphy Memorial Veterans Administration Hospital. From 1 July, 1966 through 30 June, 1970 he was a resident in surgery at the University of Colorado.

The gallbladder as a conduit between the liver and intestine

Surgery, 74: 525-9, 1973

William R. Waddell and Frederick L. Grover

The use of the gallbladder as a connecting conduit bewteen the pro imal bile duct system and the intestine has been described and illustrated by patients with traumatic injury of the bile ducts and inoperable carcinoma of the pancreas in whom this method was utilized. It is suggested that some of the problems of bile duct reconstruction after orthotopic liver transplantation might be amenable to solution by this technique.

The gallbladder is commonly used to allow biliary drainage into the intestine. Its accessibility is advantageous. However, there are sometimes difficulties.The cystic duct may be very narrow or actually stenotic from previous inflammation. The valves of Heister are a potential source of obstruction. Tumor may encroach upon the cystic duct or be so near as to make it unacceptable when attempting palliation. Trauma or infection may have destroyed the segment of the common bile duct from which the cystic duct arises. Also for the special needs of hepatic transplantation and reconstruction after Whipple operations, a low entry of the cystic duct may preclude use of the gallbladder if the cystic duct is to be used as a connecting conduit between the common bile duct and the gallbladder.[2-4] Even if bile and contrast material drain into the intestine via the cystic duct and gallbladder, there may be back pressure.

Methods

To circumvent these and other difficulties, the infundibulum of the gallbladder has been anastomosed to the right hepatic duct on one occasion and the common hepatic duct in three cases (Fig. 1). These procedures were straightforward and the functional effect seems to be excellent. Recent reports from transplant surgeons[2-4] indicate that there may be a wider use for this approach to the problem of bile duct reconstruction and other possible applications are suggested by the following four cases which illustrate the actual and potential utility of this procedure. A description of the previous use of this exact procedure has not been found, but a recent report by Javara and associates[3] describes creation of a tube from the gallbladder. The presently described technique is less complicated and appears to be the same functionally.

Case Reports

Case 1 (C.G.H. No. 333643). A 16-year-old female was admitted to Colorado General Hospital in December, 1968, shortly after an automobile accident in which she was thrown against the dashboard. Because of mild shock and signs of peritoneal irritation a laparotomy was performed and a ruptured spleen was removed. The only other abnormality was a retroperitoneal hematoma involving the tissues about the duodenum. A Kocher maneuver and careful inspection of the area failed to reveal the source of bleeding that had caused the hematoma. A drain was placed into the dissected area.

Twenty-four hours later bile began to drain to the exterior. Radiologic studies failed to clarify this situation and accordingly the patient was returned to the operating room for a second exploratory operation. The bifurcation of the common hepatic duct was avulsed from the right and left hepatic ducts. This area was reconstructed over a No. 12 latex rubber Y tube (Fig. 2). From this time on, the convalescence period was normal. After preliminary clamping and radiologic evaluation, the Y-tube was removed approximately two months after the original injury.

A month later, jaundice, chills, fever, and right abdominal pain led to readmission and another laparotomy for repair of a stricture involving the proximal portions of both the right and left hepatic duct and 3 cm. of the upper part of the common hepatic duct. The solution to this problem was first to mobilize the distal common duct and carry out an end-to-end suture of this to the dilated left hepatic duct over a T tube (Fig. 3). The original intention in this case was to rejoin the ducts in the normal fashion. However, that plan had to be abandoned when it was found that the right and left ducts could not be brought together because of tissue loss, scarring, and infection. The right hepatic duct was anastomosed to the gallbladder (Fig. 4). The second alteration in the course came when it was found that contrast material could not be forced from the gallbladder into the common bile duct (Fig. 4); therefore the cholecystoduodenostomy was constructed. These various anastomoses were all made with inner rows of catgut and the outer rows of fine silk (exterior layers only). A straight catheter was passed

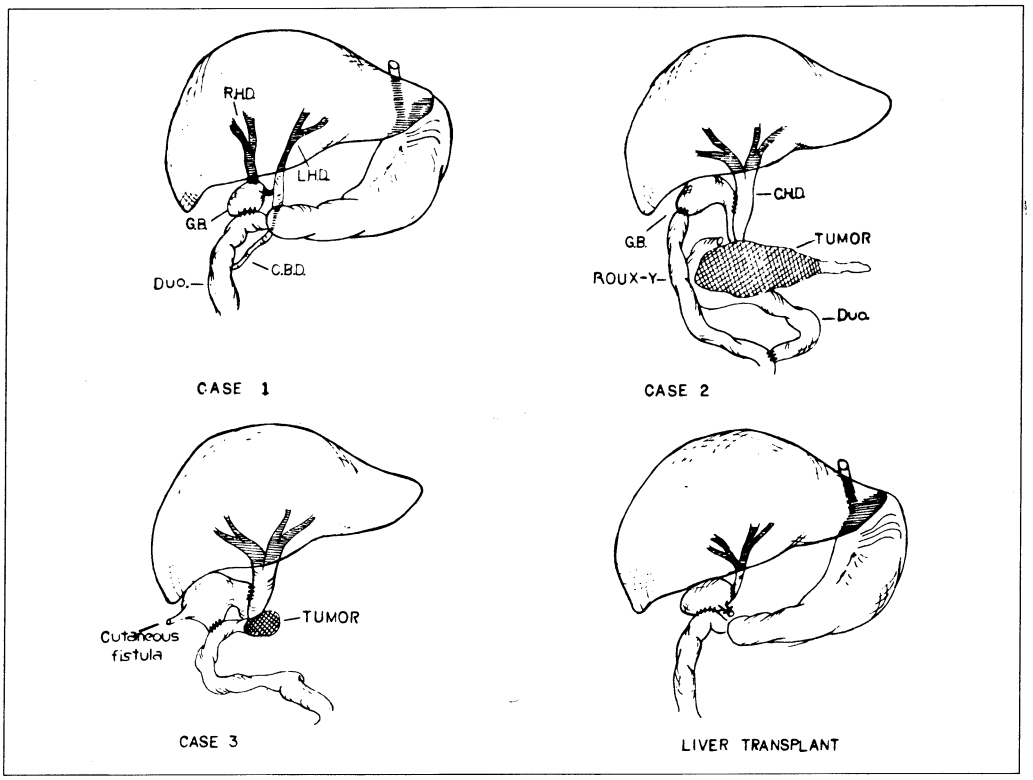


Fig. 1.— Diagrammatic illustration of the anatomic arrangement of structures and the bile drainage systems in three cases described and a suggested method of bile drainage for orthotopic liver transplant.

through the gallbladder into the right hepatic duct system. Fig. 1 shows the anatomical arrangement after reconstruction. Convalescence was uneventful. The tubes were removed after three months. The patient has remained well until the present, which has been for 3 1/2 years.

Case 2 (C.G.H. No. 455652). A 52-year-old electrician developed jaundice and vague upper abdominal symptoms in January, 1972. Laparotomy disclosed a large adenocarcinoma of the head and body of the pancreas. It was inoperable because of local extension into the retroperitoneum and about the superior mesenteric vessels. A Roux-en-Y loop of jejunum was anastomosed to the gallbladder. The jaundice cleared rapidly, and the patient was given weekly intravenous injections of 5-fluorouracil. He had mild upper abdominal pain and discomfort but was generally well for the next nine months when jaundice reappeared and rapidly deepened. Liver function as determined in serum samples was consistent with extrahepatic biliary obstruction.

In October, 1972, another laparotomy showed a contracted hard pancreas with no evidence of hepatic or lymph node metastases. The distal common bile duct and the origin of the cystic duct were surrounded by tumor and probably scar tissue as judged by the contracted and shrunken mass in the vicinity. The common bile duct was 2 cm. in diameter. The gallbladder lay adjacent to it. The cholecystojejunostomy was widely patent. A large anastomosis between the gallbladder and the common hepatic duct was constructed (Fig. 1). The patient's jaundice cleared rapidly, and after a short time he recovered and was discharged to resume chemotherapy in the outpatient clinic. He has remained well to date.

Case 3 (C.G.H. No. 413994). A 44-year-old housewife developed obstructive jaundice for which laparotomy was performed in June, 1971. A lesion compatible with carcinoma of the head of the pancreas involving the distal common bile duct and portal vein was found. A choledochoduodenostomy and cholecystostomy were performed. In August, 1971, another laparotomy was performed to obtain a histological diagnosis, and at the same time, a gastroenterostomy was established to relieve partial duodenal obstruction caused by encroachment by a large carcinoma of the pancreas which appeared to replace the entire pancreas. Only the midportion was biopsied, and it showed adenocarcinoma.

Shortly after this operation, the patient was begun on 5-fluorouracil and testololactone. This has been continued to date. Her principal difficulties have been due to attacks of cholecystitis and possibly cholangitis as long as the cholecystostomy tube remained in the gallbladder. It finally fell out. After one year of weekly administration of 500 mg. of 5-fluorouracil, the dosage was reduced to 450 mg. per week.

In October, 1972, during a flu epidemic that severely affected the patient and her family, she developed diabetes mellitus. The insulin requirement was 50 U. per day. Fluorouracil and testololactone were continued after a brief trial of aldactone which was tolerated poorly. In late December, 1972, she became deeply jaundiced until the previous cholecystostomy site opened and drained large quantities of bile. After reevaluation of gastrointestinal and biliary tracts by radiologic examination, it was apparent that the duodenum, the distal common bile duct, and the choledochoduodenostomy were obstructed. At laparotomy in January, 1973, the pancreas was represented by a 3 by 4 cm mass, the right-hand extremity of which encroached upon the common duct and the first part of the duodenum. The mass was very much smaller than 18 months previously, and most of the pancreas seemed to have disappeared, leaving the duodenal loop collapsed with the second portion overlying the spine and aorta. An anastomosis between the gallbladder and proximal common hepatic duct and then a side-to-side cholecystoduodenostomy were made (Fig. 2, Case 3). Her convalescence was uneventful, and she had resumed

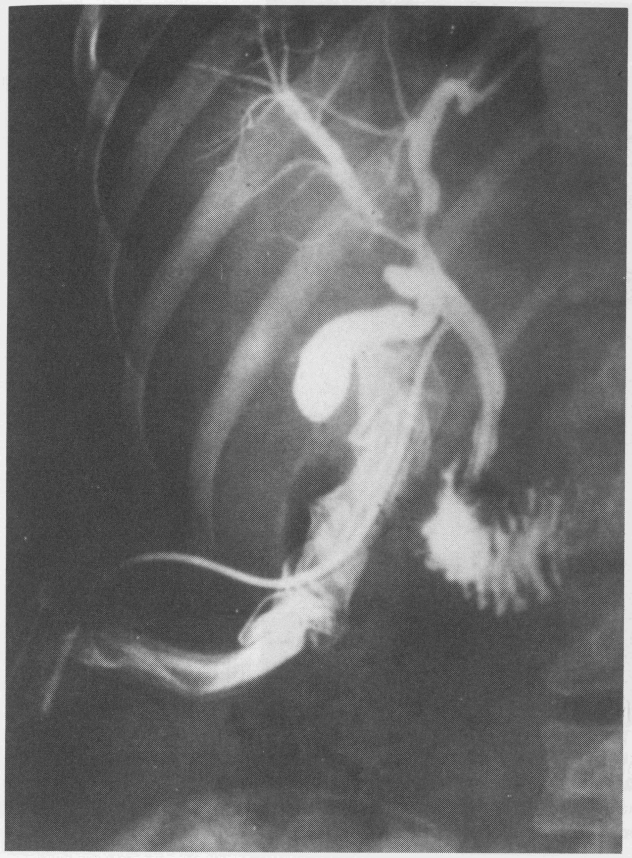


Fig. 2.— Cholangiogram in Case 1 showing Y tube in place and the damaged bifurcation and hepatic duct.

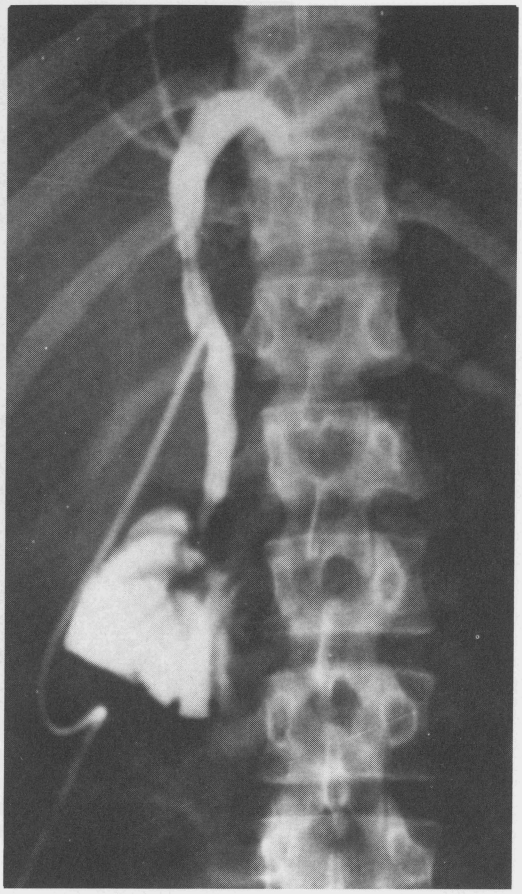


Fig. 3.— Cholangiogram in Case 1 showing reconstructed left hepatic duct anastomosed to common hepatic duct over a T tube.

her usual state of health while receiving 5-fluorouracil, testololactone, insulin, and pancreatic enzyme replacement.

Case 4 (C.G.H. No. 468033). A 40-year-old engineer became jaundiced in January, 1972. He had no symptoms. He had taken large quantities of alcohol daily for years, but he had not been ill as a result and was not considered to be addicted to alcohol. A month later, a laparotomy was carried out at which time a distended gallbladder, obstructed common bile duct, and nodular hard pancreas were found. The duodenum was opened and biopsies of the ampulla of Vater and pancreas were obtained. No tumor was identified in these specimens. A loop cholecystojejunostomy was constructed and below this the two limbs of jejunum were joined with a jejunojeunostomy.

During the subsequent nine months, the patient continued to be without symptoms and the jaundice cleared. In November, 1972, he began to have upper abdominal pain, anorexia, nausea, vomiting, and weight loss. A variety of laboratory tests and radiologic examinations failed to reveal the cause of severe and worsening symptoms. Gastric hypersecretion to the extent of 2 L. a day and high acid content (80 to 90 mEq. per liter) were the only significant additional data. The patient was transferred to the University of Colorado Medical Center where a short while later another laparotomy was performed. The patient had carcinoma of the pancreas almost completely obstructing the second part of the duodenum. There was implantation of tumor in the previous duodenostomy site, and the porta hepatis was involved to some extent. Although the cystic duct was not obstructed, its origin was at the upper margin of the main tumor mass. There was a 5 cm. cyst in the midportion of the pancreas. Vagotomy and gastrojejunostomy, pancreatic cystgastrostomy, and cholecystocholedochostomy were carried out. The latter anastomosis was between the gallbladder and the common bile duct immediately below the junction of the main ducts from the right and left lobes (as in Case 2, Fig. 1).

Convalescence from this operation was uneventful. The patient's symptoms, mainly caused by duodenal obstruction, were relieved. He has been maintained on chemotherapy for two months and has returned to work. He is improving steadily and a long period of control of cancer is anticipated.

Discussion

As illustrated above, palliation of inoperable carcinoma of the pancreas, bile duct reconstruction after trauma and perhaps hepatic transplantation are possible uses to which this procedure might be put. The procedure has certain advantages that can be mentioned. Both of the anastomoses are relatively easy because they can be made large and there is no tension. Contrary to many illustrations, the infundibulum of the gallbladder is usually alongside or very near the common hepatic duct. The inaccurate portrayal of the exact anatomy of the region probably has resulted from spreading things out too much to avoid overlap. There is naturally some variation but generally the right hepatic duct and the upper common hepatic duct are in juxtaposition to the gallbladder.

In connecting a normal-sized common bile duct or common hepatic duct to the gallbladder, as in reconstruction after orthotopic liver transplantation, a duct with a long beveled end opening or a linear side opening will allow the creation of an anastomosis which is considerably larger than the actual and functional diameter of the duct. In anastomosing a distended common hepatic duct to the gallbladder, a simple side-to-side connection has been used as illustrated. Another anatomical consideration is that the common hepatic duct immediately below the bifurcation is about as far

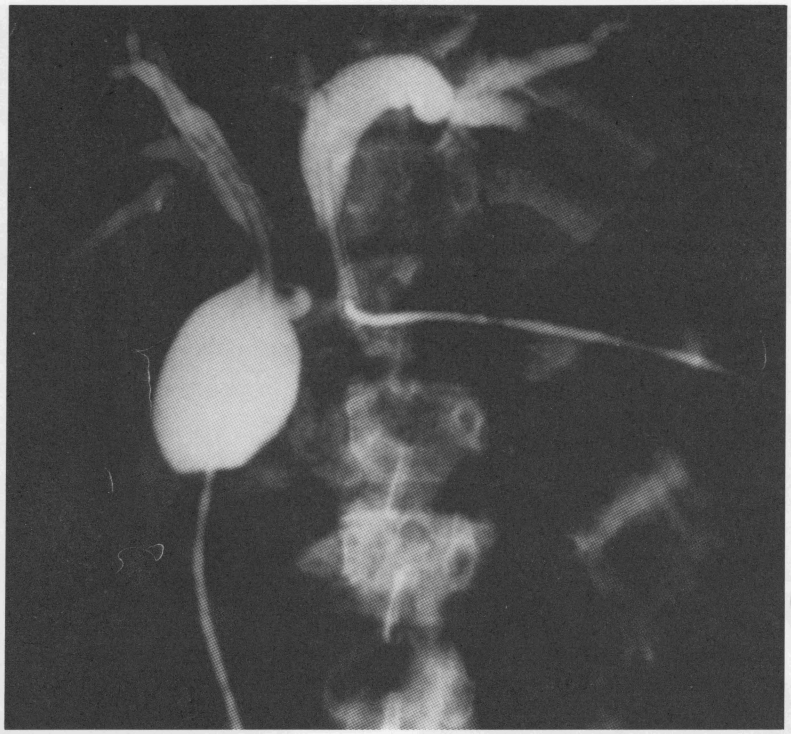


Fig. 4.— Operative cholangiogram in Case 1 showing left hepatic duct anastomosed to the common hepatic duct over a T tube and the right hepatic duct anastomosed to the gallbladder with a straight catheter through the anastomosis. Note that despite distention of the gallbladder, no contrast material enters the common bile duct. Subsequent to and because of this demonstration, the gallbladder was anastomosed to the duodenum.

from tumor in the head of the pancreas as one can place an anastomosis. It is believed that this might prolong the interval between operation and extension of tumor to cause recurrent biliary obstruction (Fig. 1, Cases 2, 3, and 4).

Finally, if this type of reconstruction were adopted for hepatic transplantation, it would have the following advantages. First, the catastrophe of utilization of a cholecystoduodenostomy when a low entering cystic duct has been ligated, divided, or otherwise obstructed can be avoided. There have been several such cases. Second, the avoidance of reverse flow through the cystic duct will eliminate extrahepatic obstruction at the cystic duct level from rejection and bacterial or viral infection. Third, no tubes or drains are necessary in this type of reconstruction; because the anastomoses are large and accessible, they can be made watertight and do not require stinting or temporary decompression of the proximal or distal structures. Fourth, this procedure is advantageous in allowing the proximal anastomosis to be made with two openings the same size with tissues of approximately the same thickness. Similarly, the gallbladder-to-intestine anastomosis can connect two openings of the same diameter. This in contrast to methods in which a small bile duct is anastomosed to the intestine.

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This was a relatively complete account given at the American Surgical Association of the first nine years of the Cambridge-King's College liver transplant program. Immunosuppression was with azathioprine and prednisone. Calne had adapted the procedure for biliary reconstruction originally developed by Waddell for routine use in his liver transplant recipients. Thereafter biliary tract complications were reduced greatly in the Cambridge experience.

Observations on preservation, bile drainage and rejection in 64 human orthotopic liver allografts

Annals of Surgery, 186: 282-90, 1977

R. Y. Calne, P. McMaster, B. Portmann, W. J. Wall and Roger Williams

The combined Cambridge/King's College Hospital series of 64 orthotopic liver grafts' experience dates back to 1968. Many patients were referred for liver grafting late in the course of their diseases and were operated on when they were too ill or died before suitable livers could be found. Complications of biliary drainage were the most frequent causes of death. In the past two years we have acquired experience of a method of liver preservation which permits up to 8 hours of safe storage without any complicated machines and we have been able to transport 22 livers by air and road from other institutions to our own unit. This has enlarged the pool of potential donors and therefore reduced the average waiting time for a liver transplant. A new method of biliary drainage has been employed in 24 patients using the vascularized gall bladder as a conduit between the donor and recipient common ducts. This has resulted in a marked reduction in early postoperative complications of biliary fistula and obstructed bile drainage. Now that survival has improved, it has become clear that uncontrollable rejection of the allografted liver in man is much less severe than that experienced with kidney transplants. The results of liver grafting in our unit during the past year are better than those obtained with kidneys from cadaver donors.

We have recently published overall results on the first 60 patients receiving orthotopic liver allografts in the Cambridge/King's College Hospital series.[1] There has been a steady improvement and many errors previously made have been recognised and are now avoided. It is the purpose of this paper to describe the current changes in management of patients, to discuss liver preservation, biliary drainage and the low incidence of rejection of liver allografts.

Indications and Overall Results

In theory, anyone dying from disease primarily confined to the liver would be a potential candidate for liver transplantation. The two disease categories that need consideration are non-neoplastic parenchymatous liver disease, in particular most forms of cirrhosis, and primary malignancy of the liver. The results of liver grafting for metastatic tumor have been poor due to early tumor recurrence. Preoperative assessment is seldom straightforward. The prognosis of parenchymatous liver disease is notoriously variable and the timing of the operation is of great importance. Liver grafting should be considered the first day it is clear that the patient will not be discharged from hospital. It is useless to wait until the patient is moribund with hypostatic pneumonia, since there is no adequate artificial substitute for liver function that can improve the patient's condition. Primary malignancy of the liver should be treated by partial hepatic resection when this is possible. If, however, both lobes are involved liver transplantation may be considered. Australia antigenemia is not a contraindication to transplantation, although rigorous precautions are required to prevent disseminating infection. One of our patients with Australia antigenemia and a hepatoma received hyperimmune gamma-globulin follow-

TABLE 1. *Indications for Orthotopic Liver Grafting*

Primary cancer of liver and bile ducts		34
Hepatocellular carcinoma	20	
Cholangiocarcinoma	12	
Kupffer cell sarcoma	1	
Haemangioendothelioma	1	
Metastatic cancer of the liver		4
Cirrhosis		22
Cryptogenic	7	
Primary biliary	4	
Alcoholic	3	
Post hepatitis	2	
Secondary biliary	2	
Subacute hepatic necrosis	1	
Sclerosing cholangitis	1	
Haemachromatosis	1	
Galactosaemia	1	
Budd-Chiari syndrome		2
Extrahepatic biliary atresia		2

64 patients treated 2nd May 1968–7th March 1977.

TABLE 2. *Survival of 64 Recipients of Orthotopic Liver Allografts*

	Total Performed	Died Within First Week	Died Between First Week and Six Months	Died Between Six Months and One Year	Survived More Than One Year	Alive and Date of Allograft
1968–1974	35	11	19	2	2 (5 yr 3 mth) (2 yr 1 mth)	1 (19 Feb 74)
1975–March 1977	29	0	10	4	1 (1 yr 1 mth)	14 (24 Dec 75) (30 Dec 75) (12 Apr 76) (20 Apr 76) (3 Oct 76) (8 Nov 76) (10 Nov 76) (19 Nov 76) (17 Dec 76) (29 Dec 76) (3 Feb 77) (20 Feb 77) (20 Feb 77)
	—	—	—	—	—	— (7 Mar 77)
Total	64	11	29	6	3	15

ing removal of the diseased liver, before the graft was revascularised. His blood is now free from Australia antigen more than a year after operation.[5] The prognosis of acute liver failure is difficult to predict. Fatal downhill progress is usually rapid and liver grafting is therefore unlikely to be suitable treatment. Infection is contraindication to liver transplantation. Most patients who have received liver grafts, when there has been established infection in any part of the body prior to surgery, have died from progressive, uncontrollable sepsis.

Thirty-eight of our patients suffered from malignancy of the liver, four of these had metastatic liver tumor. Twenty-six patients had non-malignant liver disease, cirrhotic processes consituting the majority (Table 1). The median age of the patients was 43 years, the oldest 65. Only five patients were aged 17 or under. One was aged under 11 years, an infant of ten months with biliary atresia. Of the 35 patients operated on before 1975, 11 died within the first week after operation but there were no deaths within this period in the 29 cases operated on from 1975 and 14 of these 29 patients are alive. Six patients lived more than a year. Of the 15 patients currently alive, one has survived more than three years after operation. The longest survivor in the series lived five years and three months (Table 2). Although a variety of complications may in each individual case contribute to a fatal outcome, the primary cause of death as shown in Table 3 was most frequently due to complications of biliary drainage. Recurrent tumor and sepsis in the presence of satisfactory biliary drainage were next in the frequency. Unmanageable rejection was responsible for death in only four patients. The improved peri-operative survival since 1975 is believed to be due to increased experience in the anesthetic management of the patients during operation and better quality donor organs. During the operation great care is taken when the vena cava is clamped above the liver to ensure that there is sufficient infusion of blood to the superior vena cava since the return of blood to the heart may be reduced by more than 50%. Prior to revascularisation of the liver, the organ is flushed through the portal vein with 400 ml of plasma protein fraction at room temperature to remove potassium ions and acid metabolites. It is important that there should be a continuous dialogue between the surgeon and the anaesthetist so that major cardiovascular and metabolic changes are anticipated and corrected before serious abnormalities occur. All blood transfused to the patient is filtered. General anesthesia is supplemented by an epidural block, which is continued postoperatively. This enables the patient to sit up, inspire deeply and cough during the early postoperative period. We believe this has reduced the incidence of postoperative pulmonary complications.

Liver Preservation

Removal of the liver from a "heart beating cadaver" provides the most perfectly preserved organ for transplantation. Until recently in the United Kingdom, where the concept of brain death had not been accepted, organ removal was started when circulatory arrest had occurred following cessation of mechanical ventilation. The liver suffered a severe handicap imposed by the ischaemic damage occurring while the heartbeat continued perfusing the organ with increasingly deoxygenated and acidic blood. In the past two years it has been possible to commence liver removal, either immediately after cessation of mechanical ventilation or while ventilation continued. Fifteen of the last 21 livers transplanted have been removed form "heart-beating cadavers." The initial cooling of the liver is performed in situ through the portal vein with gravity drainage of Hartmann's solution at 4°. After infusion of 1.5-2 litres of this solution the flush is changed to

TABLE 3. *Primary Causes of Death and Other Serious Complications*

Deaths in first postoperative week		11
Hemorrhage	6	
Cardiac arrest	3	
Pneumonia	1	
Acute Rejection	1	
Deaths from complications of biliary drainage		13
Deaths from recurrent tumor		9
Deaths from sepsis in the presence of satisfactory biliary drainage		8
Deaths from chronic rejection		3
Other causes of death:		5
Thrombosis of IVC and portal vein	2*	
Hepatic artery thrombosis	1	
Spontaneous colonic perforation	1	
Myocardial infarction	1	
Other serious complications:		
Reticulum cell sarcoma	In patient dying after 6 months from sepsis	
Operative cardiac arrest successfully resuscitated	In patient surviving more than 3 years	
Development of adenocarcinoma of rectum	In patient dying after one year from recurrent cholangiocarcinoma	

* One of these patients had suffered from the Budd-Chiari syndrome and was not anticoagulated postoperatively.

an ice-cold protein fraction with additives.* Seven hundred milliliters of this solution are infused through the portal vein and another 300 ml via the hepatic artery. The liver is then stored in ice-cold saline in a sterile container surrounded by ice.[14] Since 1973, 22 livers have been preserved by this method and transported to Cambridge or King's College Hospital from institutions between 28 and 200 miles away. The ischemia times have ranged from two hours to seven hours 50 minutes. All the recipients had good or excellent postoperative function of their liver grafts. With such a simple and satisfactory short-term preservation technique we doubt whether there is a need for complicated perfusion apparatus. The shortage of liver donors has necessitated the use of extremely poorly matched donors. This does not seem to have affected adversely the results of clinical liver grafting. Indeed, grafts have functioned well in the presence of strong specific anti-donor cytotoxic antibodies and when A, B, O incompatible donor livers have been transplanted.[11] To transplant kidneys with similar, apparently overwhelming, immunological disadvantages would be courting disaster.

Bile Drainage

This is the technical Achilles' heel of liver transplantation. Approximately 50% of our patients and those in the Denver series have developed serious biliary tract complications and this figure would probably be higher if patients succumbing in the earlier postoperative phase had survived longer. Direct duct-to-duct drainage may fail because of a poor supply to the lower end of the donor common duct and tension on the anastomosis. The protective defence of the sphincter of Oddi against infection is however retained. In experimental animals if the sphincter of Oddi is bypassed cholangitis is an almost invariable sequel. This complication has been common in man when bile drainage has been into the duodenum bypassing the sphincter. Techniques utilising the gall bladder have had the disadvantages of the narrow cystic duct which may become blocked and the blind sump end of the common duct distal to the entrance of the cystic duct, in which bile sludge may accumulate.

Since November 1973 Starzl has favoured using a long Roux loop to avoid ascending infection and anastomosing this to the gall bladder or the common bile duct.[11] We feel that the objective of biliary drainage should be to preserve the sphincter of Oddi, to provide a wide anastomosis with a good blood supply, to have no sump for the accumulation of biliary sludge and to have ready access to the biliary ducts for irrigation and diagnostic radiography. These objectives are obtained with a new gall bladder conduit technique that we have used in 24 of the last 29 cases.[1] In two patients the conduit was constructed as a secondary procedure after breakdown of duct-to-duct anastomoses. The remaining 22 conduits were the primary method of drainage. The gall bladder is mobilised leaving its blood supply intact. The donor common duct is trimmed back and cut obliquely to provide a wide anastomosis and a good blood supply. Hartmann's pouch of the donor gall bladder is anastomosed to the donor common duct. The fundus of the gall bladder is anastomosed to the obliquely cut recipient common duct. The anastomoses are splinted with a specially constructed T-tube with a narrow gauge irrigating arm, threaded through the proximal anastomosis (Fig. 1). One to two litres of heparinised normal saline are infused by a drip set through the irrigating tube each day for the first ten days after operation. If cholangiography (Fig. 2) is then satisfactory, further flushing is stopped and the T-tube is spigotted. It had been our policy to leave the T-tube in

* To each 800 ml of PPF is added: 2000 IU Heparin, 250 mg Hydrocortisone, 500 mg Ampicillin, 6 ml 0.1 N HCl, 5 ml. of 10% Magnesium Sulphate, 250 mg Dextrose, 15 mEq Potassium Phosphate.

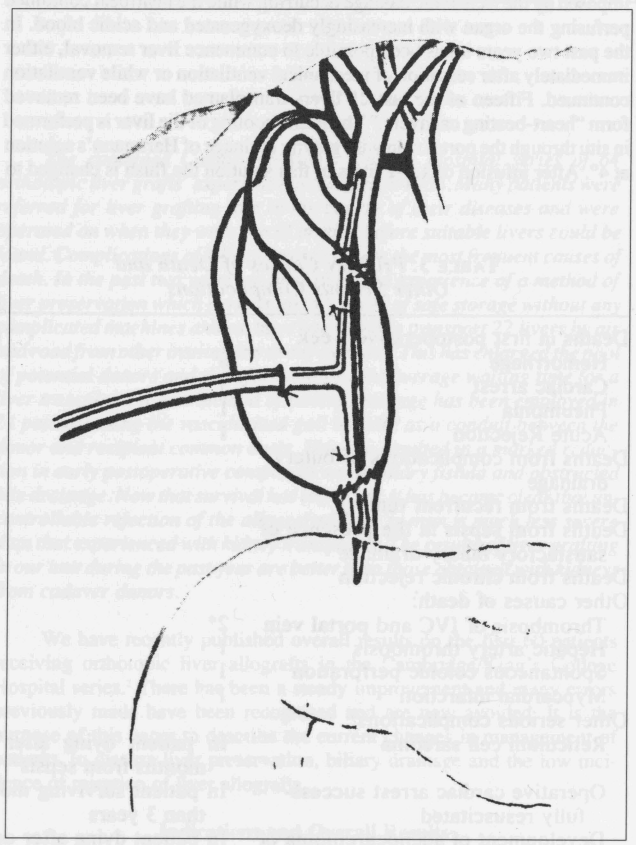

Fig. 1.— Pedicle graft conduit with donor gall bladder. Hartmann's pouch is anastomosed to donor common duct and fundus anastomosed to recipient common duct. Irrigating T-tube is inserted with irrigating arm through upper anastomosis. Blood supply to gall bladder is carefully preserved. (By courtesy of the Br. Med. J.)

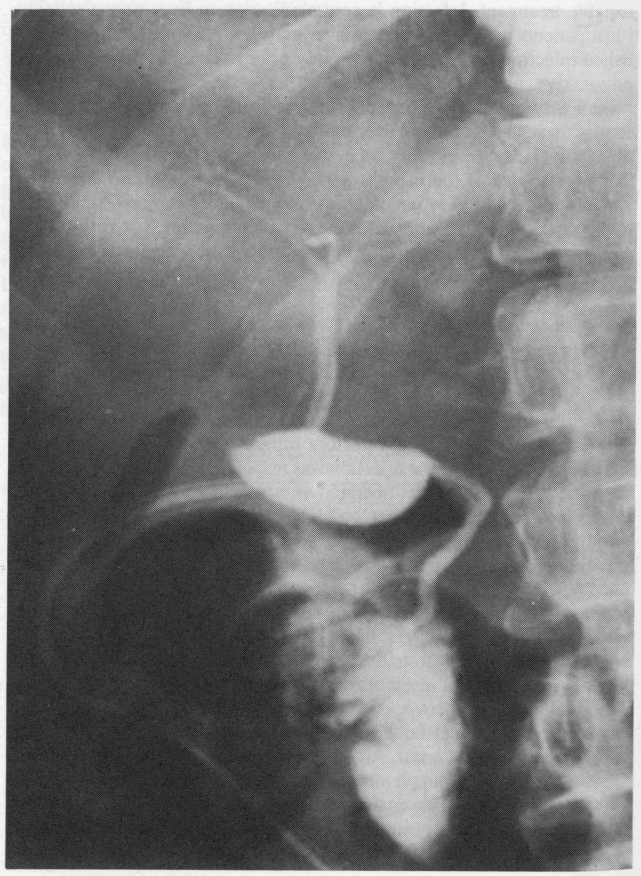

Fig. 2.— T-tube cholangiogram of patient with orthotopic allografts with gall bladder used as pedicle graft conduit. (By courtesy of the Br. Med. J.)

TABLE 4. *Complications of Biliary Drainage in 53 Patients Surviving the First Post-operative Week*

Technique	Number of Cases at Risk	Number With Complications	Biliary Fistula	Blockage by Sludge	Deaths
Duct-to-duct	18	11	8*	5	4
Gallbladder to common duct	8	5	2	5	5
Gallbladder to intestine	5	4	4	2	
	31	20	14	12	11
New gallbladder conduit technique	20	6	2	3	2
Conduit to Roux loop	2	1	0	0	0
	22	7	2	3	2
Totals	53	27	16	15	13

* Two fistulas closed by conduit technique.

place without disturbance provided there were no complications. In two patients the tubes fell out after a month. One tube has been in place for more than a year.

In view of the danger of infection and obstruction of drainage by the tube we now believe that the T-tube should be removed after three months if cholangiography shows adequate drainage. Although the follow-up is short, in each patient biliary drainage has been initially satisfactory and the incidence of biliary leakage and obstruction of bile flow has been markedly reduced (Table 4). In two patients with secondary biliary cirrhosis in whom the recipient's common duct could not be used, the technique was modified by anatomosing the fundus of the gall bladder conduit to a long Roux loop of jejunum (Fig. 3). Of the seven duct-to-duct anastomoses in the last 28 cases, two were successfully converted to conduits following fistula formation, one became blocked with sludge, the remaining four drained satisfactorily. Two recent cases had duct-to-duct anastomoses because of gall-stones in the donor gall bladder. In one case the gall bladder had been removed a year previously and in the other, stones were found when the liver was removed and a cholecystectomy was therefore performed. Our longest surviving patient had satisfactory duct-to-duct drainage for three years, but the T-tube required changing at operation after 16 months. In view of the high complication rate of other methods of biliary drainage, we would favor the conduit technique if this is possible, with a duct-to-duct as the next best biliary drainage available.

Techniques involving anastomosis of the common bile duct can be difficult, since there are marked variations in calibre of normal human common bile ducts; in adults we have seen ducts of 2 mm in diameter which accommodate only a small probe and in order to perform a satisfactory anastomosis the surgeon may require loop magnifying glasses. At the other extreme ducts may be as large as 1 cm in diameter.

Biliary Sludge

If bile drainage is interfered with in any way, there is a likelihood of cholangitis and precipitation of biliary constituents forming sludge. This secondary sludge formation has been observed frequently in recipients of liver allografts with impaired biliary drainage.[12] There is another form of biliary sludge which usually presents in the first or second postoperative weeks as the only demonstrable cause of obstructed bile drainage. A major constituent of these cases is collagen (Fig. 4). Cholesterol and bilirubin are present in relatively small amounts.[6] The incidence of this primary sludge is not known since it has only recently been recognised. It produced biliary obstruction in at least six patients. The shedding of collagen from the lining of intra- and extrahepatic ducts probably follows ischemic necrosis. This damage may be incurred in the transplant operation, but could also be the result of rejection. In the pig the biliary duct epithelium does not appear to be more aggressively rejected than the rest of the liver parenchyma, but primary biliary sludge has not been observed in porcine liver allografts and we have little information on the susceptibility of bile duct epithelium to rejection compared with the rest of the liver parenchyma in man (*vide infra*). Primary sludge can also occur late when there has been occlusion of the arterial supply to the liver. It was observed after five years in one of

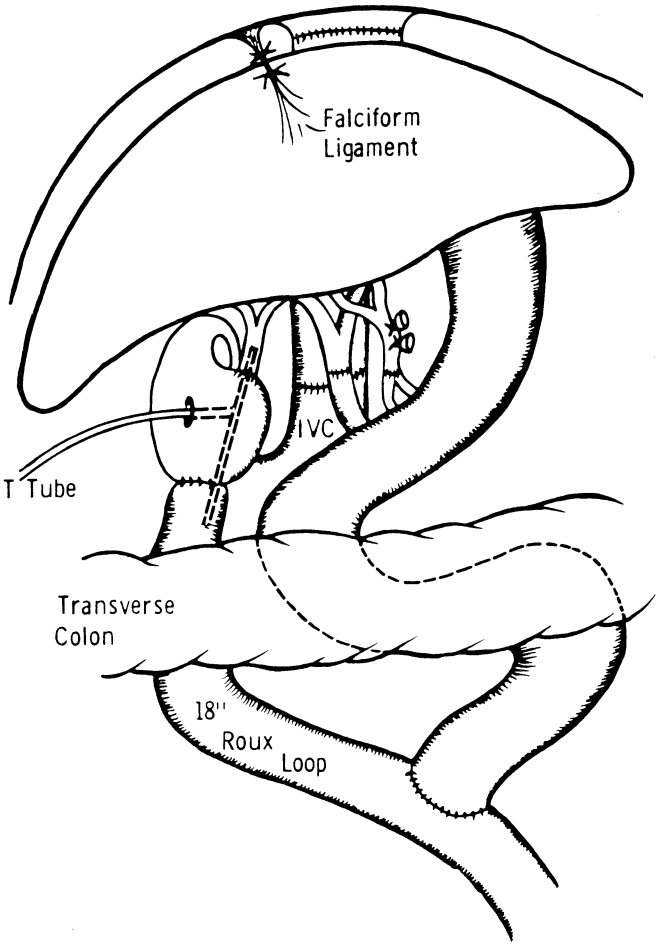

Fig. 3.— Gall bladder conduit technique used when the recipient common bile duct is unsuitable. The fundus of the gall bladder is anastomosed to a long Roux loop of jejunum. (By courtesy of the World J. Surg.)

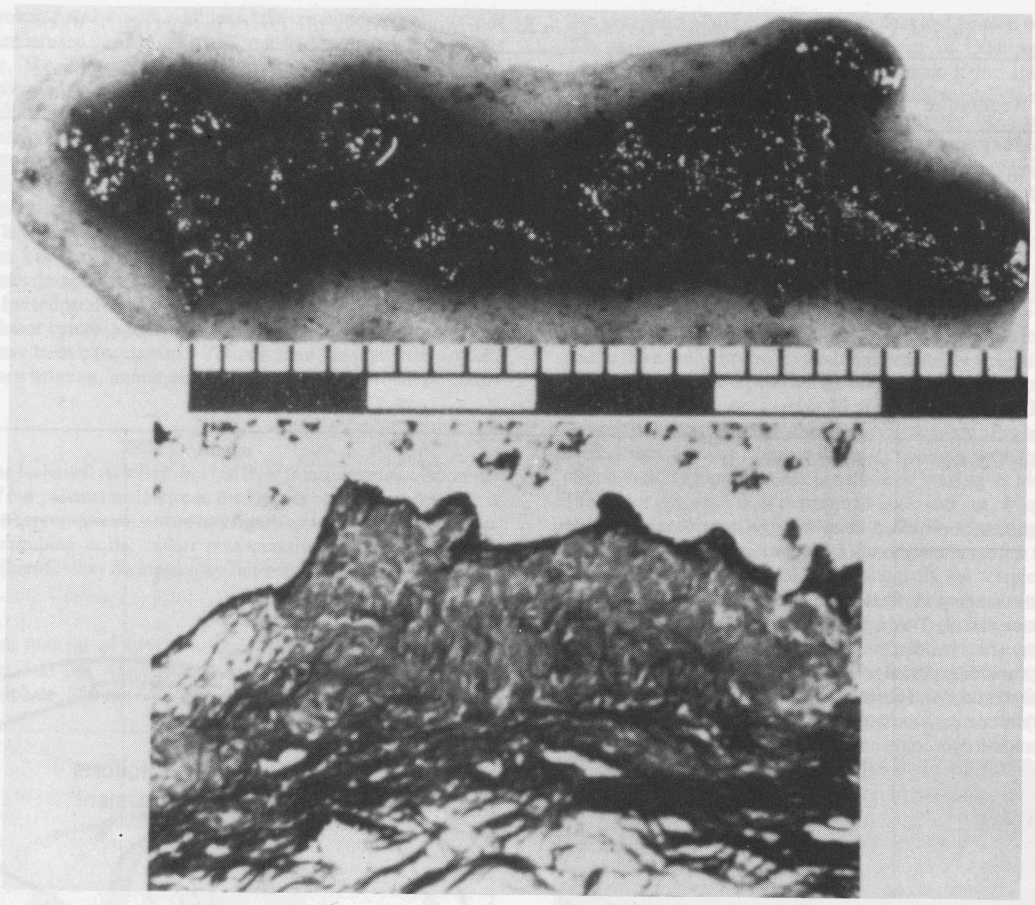

Fig. 4.— Morphology of primary biliary sludge. (Top) shows gross appearance. (Bottom) shows the main constituents to be collagen from bile duct wall, the architecture of which is in part preserved (hematoxylin and eosin X 160).

our patients and after a year in another. Once collagen has been liberated into the biliary duct system it cannot be dissolved, but the irrigation in the first two weeks is aimed at preventing its accumulation into obturating casts, and certainly biliary sludge has been far less common since irrigation has been used. We now also irrigate duct-to-duct anastomosis. Primary sludge has not occurred in any of the 15 transplants where the livers have been taken from heart-beating cadavers, nor has it been present in the four patients who had uncontrollable rejection. We therefore feel that the most likely culprit in the pathogenesis of primary biliary sludge is non-immunological ischaemic change. Bile secretion was investigated in three of our patients.[13] Supersaturation of bile with cholesterol was found in two cases immediately after surgery and during episodes of acute rejection. The bile was never lithogenic in the third case. Subsequent studies suggested that changes in bile composition do not influence sludge formation. We agree with the Denver group that secondary bile sludge formation is usually due to anastomotic stenosis and infection.

Immunosuppression

The standard immunosuppressive treatment has been Azathioprine and Prednisone in doses comparable to those used in renal transplant patients. In view of the ischaemic damage in many livers transplanted in our series Cyclophosphamide is now given for the first three days instead of Azathioprine. Two patients were weaned completely off Prednisone and maintenance therapy of immunosuppression in most patients has required less drug dosage than patients with kidney grafts. In contrast to the Denver practice, we do not now use antilymphocyte globulin.

Occurrence of Rejection

Rejection can be difficult to diagnose. Needle biopsies have been performed but have been of less practical value than biopsies of renal allografts. A positive leukocyte migration test[4] in the presence of deteriorating liver function and a normal T-tube cholangiogram is assumed to indicate rejection and a short course of increased steroid dosage is commenced. This is usually followed in a few days by an improvement in liver function.

Most of the patients have shown a rise in serum bilirubin starting seven to ten days after the operation. Often there has been no other evidence of rejection at the time and the jaundice has subsided without change in immunosuppressive therapy. Although it is possible that the early rise in serum bilirubin level is related to ischemic damage, it does not appear to have become more severe or frequent since the use of livers removed at other hospitals and transported over long distances. There is no evidence that these episodes are related to azathioprine toxicity since they still occurred in cases in which cyclophosphamide was substituted for azathioprine during the first two post-operative weeks.

In four patients severe rejection was considered to have been a major cause of death. Included in these was one patient in whom immunosuppressive drugs had been withheld because it was thought at the time (1968) that the hepatitis virus, presumed to be the cause of his subacute hepatic necrosis, might be reactivated. The other three patients became deeply jaundiced with high serum alkaline phosphatase and moderately raised serum transaminase levels, these continuing until their deaths.

The final morphological changes in the allografts were mononuclear cell infiltration of the portal tracts and the presence of foamy lipid laden histiocytes in the walls of the hepatic arterioles and throughout the sinusoides. A feature that has not been previously described occurred in one of our patients, namely virtual absence of small bile ducts (Fig. 5) in the portal tracts. This may explain the relentless progressive intrahepatic

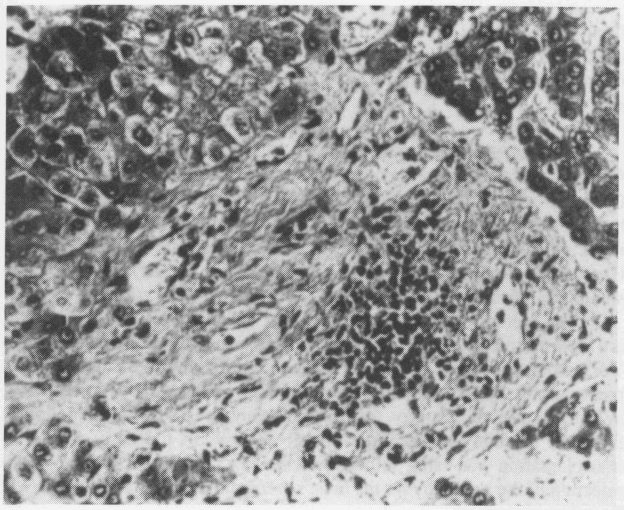

Fig. 5.— Interlobular portal tract, showing a mild mononuclear cell infiltrate and absence of identifiable duct. Post mortem liver tissue stained with hematoxylin and eosin; X 160.

obstructive jaundice that was observed with relatively good hepatic parenchymal function until just before death. His original disease was hepatoma in an alcoholic cirrhotic liver.

Discussion

Patients referred for new and dangerous surgical procedures are usually considered for operation too late in the course of their diseases. Even if the decision to transplant a liver is made when the patient is fit for surgery, his condition may deteriorate so as to constitute an unacceptable operative risk by the time a donor liver becomes available. Sepsis, tumour recurrence and above all, complications of biliary tract drainage have been responsible for the poor overall survival rate of recipients of liver grafts. Nevertheless, patients who have been discharged from hospital have often done extremely well. They frequently require less steroids than renal transplant patients and therefore do not have such severe cushingoid features. One hundred and three of the patients in Starzl's series were transplanted a year or more ago.[9] Thirty of them or 29% have survived for at least one year after liver replacement. The one-year survival figures have been improving, fluctuating in the 25-45% range. Putnam and colleagues have pointed out that if the very heavy early mortality were to continue after the first year, there would be significant cause to question the validity of the procedure. Fortunately, most of the patients who reach the one year mark continue to do well thereafter. Thus, half of all the Denver one-year survivors are still alive. 15 patients have reached the two year mark, eight have reached three years, and there have been four five-year survivors. The longest survivor in their series and in the world is now more than seven years post-transplantation. She is living at home, attending school and has normal liver function. Two other five-year survivors are also entirely well, but the fourth one died a few days short of the six year mark of chronic rejection and partial biliary obstruction.

Although tumor recurrence is common, palliation can be achieved and there is no difference in principle between treating a patient with liver cancer by grafting and giving similar palliative treatment to a patient with cancer of the lung, stomach or colon by resection of the growth. It is, however, likely that parenchymatous liver disease rather than primary malignancy will be the main indication for liver transplantation in the future.

The simple method of short term liver preservation referred to above has increased the catchment area of donor livers and therefore shortened the waiting time for patients accepted for liver grafting. The new technique of biliary drainage using the gall bladder as a conduit has produced gratifying early results, relatively free from the previous common complications.

The most remarkable aspect of liver transplantation has been the low incidence of progressive uncontrollable rejection.[10] In this respect the human experience is similar to observations made in experimental animals. Studies in the Cambridge University Department of Surgery have shown in the pig that liver cells possess less MHC surface antigenic specificities than kidney cells[3] and hepatocytes are more resistant to killing by allogeneic lymphocytes in tissue culture than are kidney cells. The difference in expression of surface antigens on liver cells could be an important factor in the relatively privileged immunological status of the liver. Hepatic metabolism and contributions of Kupffer cells could also be relevant though definitive data on these two speculations are lacking. It had been shown that six months after grafting Kupffer cells are of host origin.[7,8]

It is likely that many patients have been overtreated with immunosuppressive drugs and this has been responsible for some of the infective complications and may in theory have impaired the development of active immunosuppressive mechanisms. We now attempt to give the minimum necessary dosage of immunosuppressive drugs. Between June 1975 and May 1976 our results for liver grafting have been better than those obtained with cadaveric renal transplants. Study of the mechanisms responsible for the low incidence of rejection of human liver allografts might help in an overall understanding of the renal and cardiac grafts, and it is possible that earlier operation on patients with non-malignant liver disease could shift the liver from third to first place in the "therapeutic batting order" of organ grafting.

Acknowledgments

Liver grafting is a complicated procedure involving a large team. We are sincerely grateful to our medical, nursing and technical colleagues in our liver transplantation program. The work would not have been possible without the active and generous support from the Departments of Anaesthetics, Pathology, Clinical Biochemistry, Haematology, Bacteriology and Radiology in our two hospitals. We thank the National Blood Transfusion Service, the National Organ Matching Service, St. John's Air Wing, Eurotransplant, our colleagues in hospitals from which donor livers have been removed and the many people who have given generously of their time to help transport livers for grafting. We gratefully acknowledge generous grants from the Wellcome Trust and the Medical Research Council. We thank Mrs. Thorburne and the Department of Medical Illustration, Addenbrooke's Hospital, for the figures they prepared and the British Medical Journal for permission to reproduce figures.

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Discussion

Dr. Joseph G. Fortner (New York, New York): This is really a major contribution, one of many of Professor Calne's and as you know, he and Dr. Starzl have been the pioneers in the liver transplantation field.

I think it's really very impressive that he has 15 patients alive for variable periods of time. In the evaluation of the 64 patients that he has transplanted, the vast majority of failures are due to factors that are controllable and correctable; and I think this is the most encouraging part of this whole presentation.

Hemorrhage, intraoperative cardiac arrest, and problems with biliary drainage are all things that we can fix; and in liver transplantation the problem is not one of some problem with rejection that we can't control, but, really, something that we can.

I must confess, however, to not being able to accept 100% the use of the gallbladder as a conduit. It sort of goes against my general surgical background to do this, and I think in a complicated setting such as a liver transplant we should use a technique that's been proven to have minimal complications in a general surgical setting.

Of the 14 liver transplants that we've done at Memorial Sloan-Kettering Cancer Center, we have found in this limited experience that an end-to-side choledochojejunostomy really is, in our hands, at least, the best way to have biliary drainage, and thus cope with one of the big problems.

We are, as you may know, investigating the heterotopic or auxiliary liver graft as an alternative to the orthotopic graft in patients who have end-stage noncancerous liver disease. We have done seven of these now. One patient is living and well more than four years after transplantation, a little boy with biliary atresia. One patient lived eight months, and one lived three and one half months.

Dr. Paul S. Russell (Boston, Massachusetts): I think all of us recognize that there are three principal problem areas that we have to face with any organ which we are trying to transplant. One is the technical, and in liver transplantation there are a number of such technical difficulties which have been called to our attention tonight.

The problem of organ availability is the second, and that is still far from solved, particularly in the case of liver transplantation. Dr. Calne and others have done quite a lot in order to improve preservation techniques, but you will note that they are still rather limited, and still far from what we can do with kidney transplantation. Thus, much more can and should be done about liver preservation.

I'd like to call special attention however, to what I think is the most interesting thing that we have heard from Dr. Calne, and that is the special quality of the liver in respect to rejection, the third problem area. I think it's true that the liver is rejected with less vigor than are many other organs, and we're gradually beginning to contruct a kind of an electromotive series of rejectability of organs, in which it may be suggested that the liver is less rejectable than is the kidney, which is less rejectable than is the heart, which is much less rejectable than is skin.

There is evidence to support this from animal systems, where we have control of immunogenetic differences and can try different organ transplants in very strictly regulated circumstances. It's interesting that this difference does not pertain with equal force when one implants just a piece of an organ as a free graft. It has to be vascularized organ, which releases its antigens into the circulation of the recipient.

Some years ago we were able to show that the liver has enzymes which will degrade antigens derived from other tissues. Whether such a mechanism is actually operative in the vascularized liver transplant or not I don't know, but I'd be very much interested to know from Dr. Calne whether he has any idea at all as to what the mechanism for the striking difference in behavior of livers on transplantation as compared to other organs might be.

Dr. Joseph E. Murray (Boston, Massachusetts): I will just make four points. I think, historically, we must give credit to Drs. Starzl and Calne for bringing clinical liver transplantation into actuality, but I believe both of the authors will recognize the experimental prototype which Dr. Francis D. Moore established in the late fifties in the laboratory at the Harvard Medical School; and I believe it was there that Dr. Calne received some of his first stimulus in the field of liver transplantation.

The hierarchy of rejection of organs, which Dr. Russell mentioned, is a definite one, and I recall, again in the mid-fifties, at a conference in New York when Professor Medawar, on reviewing some work that we had presented on the differential rejection of skin versus kidney, commented, in his own picturesque way, that the transplanters had freed the biologists from the "tyranny of the skin graft"; because in the early fifties all transplantation was using skin as the indicator organ, and, of course, the rejection of the skin is by far the most severe and the most abrupt of any of the other organs.

Interestingly enough, there is a differential rejection of kidneys from the same animal under different circumstances when it's placed into the same recipient.

I would like to make an additional comment which is not truly pertinent to Calne's report. I have never liked the use of the term "brain death". The Harvard Committe under Dr. Beecher's chairmainship which defined it didn't really use the term "brain death." They were defining death of a human being as defined by cessation of function of the brain; and I've always felt that the term "brain death" was a false one, suggesting that we surgeons were trying somehow to overlook the fact that the patient might still be alive when the orans are taken.

It's a small point, but I think we should say death of an individual, as defined by cessation of function of the brain.

And finally, we talk about long-term follow-up, and we certainly have beautiful follow-up in Dr. Calne's and Dr. Starzl's patients. In the previous paper on the ambiguous genitalia they're still looking for final results, although they have been working in the field for 20 or 30 years. I think that it behooves all of us in surgery, when we have a problem, to establish some sort of a communication system, a registry, if you wish, so that we can get good documentation which can be translated across the decades, rather than waiting for that future twenty years which always seems to be ahead of us.

Professor R. Y. Calne (Closing discussion): I take Dr. Fortner's point that the actual reason for looking for a new technique was because all the old techniques were unsatisfactory. And I'm not saying that the gallbladder conduit technique is the final answer, but in our hands it has produced much better results. I think it must wait time to be proven, but every other technique that's been used in any large series has been unsatisfactory.

I wish I would answer Paul Russell's question on the mechanism of acceptance of the liver. It's by far the most interesting aspect of liver grafting and we have been studying this in the pig for many years. We have some information, but not an answer to his question.

Liver cells, hepatocytes, have less transplantation antigens than kidney cells. They have SD-antigens, but not LD-antigens. The liver has a unique lining of the endothelium with Kupffer cells, which changed to recipient type after six months in two human cases that have been studied. This could be relevant.

We don't know if the liver has a major influence on the recipient's immune system, or whether the liver itself is less susceptible to rejection, possibly it's a combination of both these factors.

As far as Dr. Murray's comments are concerned, I'm delighted to have the opportunity to pay homage to Dr. Moore for having worked in his department, and getting my first interest in liver grafting at the Brigham, and being associated with Dr. Murray for many years in experimental and clinical transplantation. I accept also his point that brain death is the diagnosis of death of the individual.

In May, 1975, one of the editors [TES] took a sabbatical in London and with Professor K.A. Porter made a clinico-pathological assessment of 93 consecutive liver transplantations. In each case an attempt was made to find reasons for success or failure. A number of changes in management resulted including more frequent cholangiography, more frequent biopsy and an assessment of causes of hepatic dysfunction other than rejection. At the time it was not appreciated, but the stage had been set by Calne's 1977 article and by this one for the effective testing of cyclosporine when it became available several years later.

Orthotopic liver transplantation in ninety-three patients

Surgery, Gynecology & Obstetrics, 142: 487-505, 1976

T. E. Starzl, K. A. Porter, C. W. Putnam, G. P. J. Schroter, C. G. Halgrimson,
R. Weil III, M. Hoelscher and H. A. S. Reid

Liver replacement with a homograft was first attempted in a human on 1 March 1963 (29). Between then and Thanksgiving Day 1974, 92 more patients were similarly treated at our center. In this review, it will be emphasized that 27 of the 93 recipients achieved survival of at least one year after transplantation with a maximum of six years and that many of these patients have been able to return to a full and useful life. At the same time, attention will be focused upon the causes for the heavy mortality that has retarded the acceptance of this new procedure and upon means by which the record might be improved.

Methods

Material.

Pediatric patients. Fifty-six of the 93 recipients were 18 years of age or younger at the time of operation (Table I). Forty of these 56 pediatric recipients had biliary atresia. In the 36 youngest of the 40 patients with atresia, the mean age at operation was 31.3± 15.7 (S.D.) months, range 3 to 67. The four oldest children in the atresia group were seven, 11, 11 and 15 years of age. Because of their long survival, they were thought, on clinical grounds, to have intrahepatic biliary atresia, and this diagnosis was compatible with the histopathologic findings of micronodular biliary cirrhosis in their native livers. Two of these latter four livers contained incidental liver cell carcinomas. Only one such malignant condition was found in the diseased livers of the other 36 patients with extrahepatic biliary atresia in whom transplantations were carried out at a younger age.

The 16 pediatric patients with diagnoses other than biliary atresia (Table I) had a mean age of 12.9±4.6 (S.D.) years, range 1 to 18. Nine had some variant of chronic aggressive hepatitis without HB_sAG antigenemia. Three had hepatomas which could not be removed with conventional partial hepatectomy. Two had Wilson's disease. There was one example each of congenital biliary cirrhosis and cirrhosis associated with alpha-1-antitrypsin deficiency of the homozygous PiZZ phenotype.

Adult patients. The 37 adult patients treated during the same time averaged 39.0±11.1 (S.D.) years, range 21 to 68. Their most frequent diagnoses were primary hepatic malignant tumor, chronic aggressive hepatitis and alcoholic cirrhosis (Table II). All of the patients with non-neoplastic disease were profoundly ill before they were considered for transplantation. Five had a diagnosis of the hepatorenal syndrome; several were unconscious; almost all were wasted from the chronic disease.

For specific patients of either the adult or pediatric subgroup, orthotopic liver transplant or OT code numbers have been given, so that the

TABLE I.—PEDIATRIC PATIENTS

Diagnosis	No. of examples	Survival > 1 yr.	Alive* now	Follow-up period present survivors, mos.	OT Nos. of survivors
Congenital biliary atresia	40	11 (27.5)	7	13, 15, 26, 34, 46, 53, 71	91, 89, 73, 64, 53, 46, 33
Chronic aggressive hepatitis	9	3 (33)	2	13, 22	92, 77
Hepatoma, liver cell carcinoma	3	2 (67)	0	—	—
Wilson's disease	2	2 (100)	1	57	42
Congenital biliary cirrhosis†	1	1 (100)	1	43	56
Alpha-1-antitrypsin deficiency	1	1 (100)	1	25	74
Total	56	20	12	13–71	

Figures in parentheses indicate percentages.
None of the 56 pediatric patients were HB_sAg positive preoperatively.
*The eight late deaths were after 12½, 13, 13½, 14, 26, 30, 41 and 72 months; causes given in text and Table IV.
†Associated with congenital deafness.

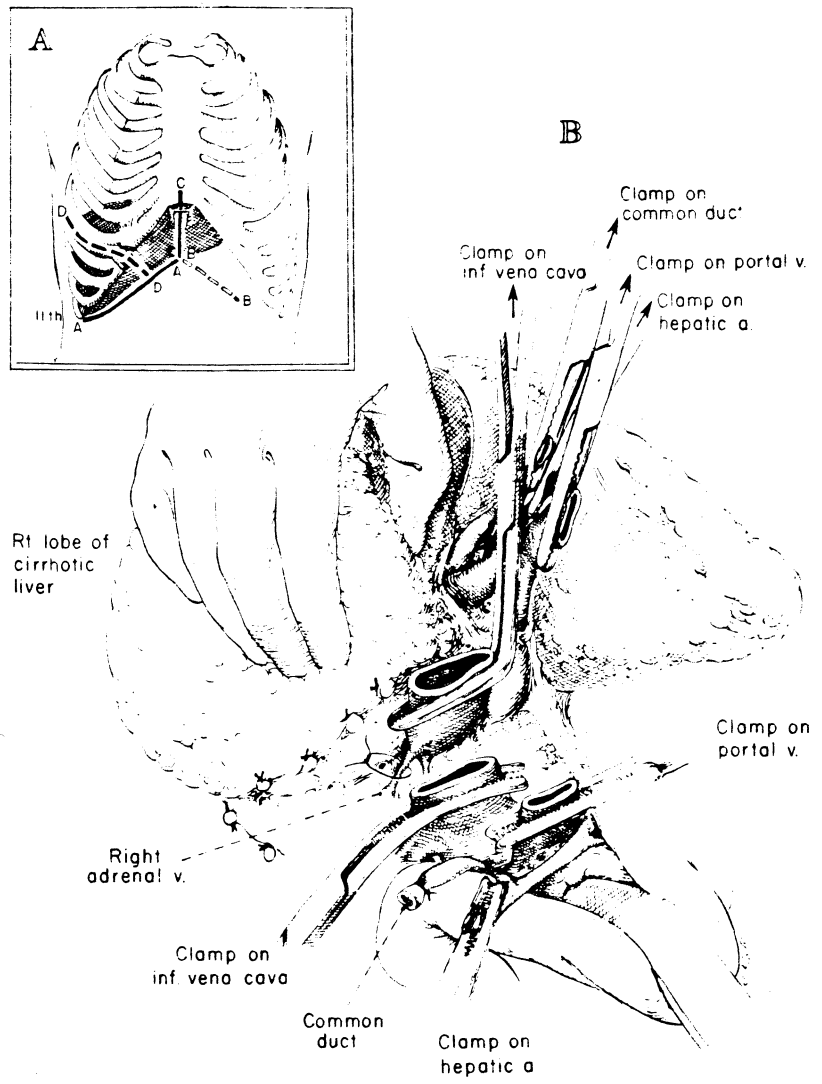


Fig. 1.— Technique of retrograde removal of liver. A, Incisions. AA, Subcostal incision used for all orthotopic liver transplantations. BB, CC and DD, Frequently used extensions from the AA incision. B, Beginning retrograde removal after transection of inferior vena cava and hilar structures. All posterior tissue that is cut should be ligated, although the named vessels encountered, such as the right adrenal vein, are few in number.

TABLE II.—ADULT PATIENTS

Diagnosis	*No.*	*Survived 1 yr.*	*Alive† now*	*Follow-up period present survivors, mos.*	*OT Nos. of survivors*
Malignant tumor*	12	2 (17)	2	15, 22	90, 78
Chronic aggressive hepatitis	9	3 (33)	0	—	—
Alcoholic cirrhosis	9	1 (11)	1	20	82
Primary biliary cirrhosis	3	0 (0)	—	—	—
Secondary biliary cirrhosis	1	0 (0)	—	—	—
Sclerosing cholangitis with ulcerative colitis	1	0 (0)	—	—	—
Massive hepatic necrosis due to hepatitis B virus	1	0 (0)	—	—	—
Budd-Chiari syndrome	1	1 (100)	1	13	93
Total	37	7 (19)	4	13–22	

Figures in parentheses represent percentages.
The patient with massive hepatic necrosis and two of the patients with chronic aggressive hepatitis had HB$_s$Ag positive tests preoperatively.
*Included seven liver cell carcinomas—three with cirrhosis—three duct cell carcinomas at the confluence of the right and left hepatic ducts which caused complete bile obstruction, one cholangiocarcinoma and one hemangioendothelial sarcoma. The two patients who are still alive had small obstructing duct cell carcinomas, but in one of these, there have been hepatic recurrences after 21 months.
†The four deaths after one year occurred after 12, 13½, 19 and 20½ months. Causes are given in text and in Table VI.

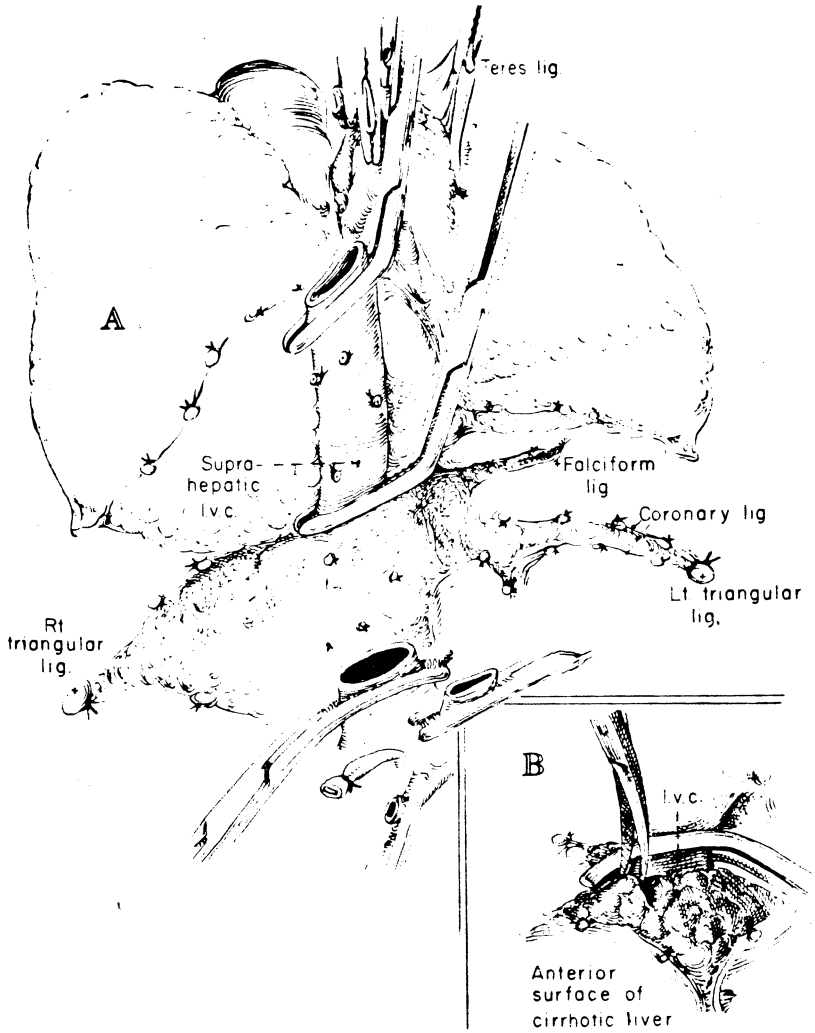


Fig. 2.— A, Operative field after retrograde liver mobilization. The last remaining structure, the suprahepatic inferior vena cava, has been clamped above the liver. B, Technique for mobilizing a suitable length of suprahepatic vena cava after placement of clamp. In adults, this usually involves cutting away cirrhotic liver tissue over the frequently distorted and foreshortened right and left hepatic veins.

reader may follow given recipients through different publications from our center. This method of patient identification has been used for most of our past publications, beginning with a major review of our experience in 1969 (31).

Management. The operative care and postoperative care of patients with liver transplantation have been described before (25, 31), but some details deserve emphasis. Since 1968 when the concept of brain death became widely accepted in the United States, cadavers usually have not been considered for liver donation unless there is some effective circulation and unless aortography can be performed to delineate the hepatic arterial supply in advance. Complicated preservation devices are no longer used. The livers of infants and children are usually perfused with 1 to 3 liters of a chilled electrolyte solution through the portal vein just before and after organ removal (31). Sometimes, the livers of adults and large adolescents may be more effectively cooled by connecting these cadaveric donors to a cardiopulmonary bypass into which a temperature reducing heat exchanger has been placed (31). Even after such preliminary total body chilling by means of a heart-lung machine, a final intraportal infusion of cold electrolyte solution is used. Incidentally, the technique of hypothermic cardiopulmonary bypass is excellent to rescue cadaveric donors who become unstable in the hours preceding the transplantation or in the course of removing the liver.

The HL-A match was not used as a criterion of donor-recipient selection, but these data were obtained in all instances since 1964. Major incompatibilities were present in all but a handful of patients (Table III). The quality of HL-A matching has not seemed to influence the outcome, although the scarcity of good matches has reduced the meaning of such a correlation.

Preformed antired cell isoagglutinins that react against donor tissues and cytotoxins, which can be detected by their lysis of donor lymphocytes, immediately destroy many renal homografts that are transplanted in violation of such positive crossmatches. The liver is resistant to this so-called hyperacute rejection (26). In our series, three liver transplantations were carried out in spite of red cell group incompatibility and three more were performed in confrontation of cytotoxic antibodies (Table III). There were no unequivocal hyperacute rejections. An account of this experience is being published separately.

Other modifications of technique or changes in our past policy should be mentioned. Originally, splenectomy was performed at the time of transplantation if this procedure had not already been carried out at an earlier time and if it was considered to be safe. Of the first 80 patients, four had prior and 57 concomitant splenectomy. In the last 13 patients of this report, splenectomy was omitted.

Host hepatectomy is usually still performed by individually dissecting the hilar structures and the vena cava above and below the liver and by then cross clamping and dividing the vessels just as the liver is removed

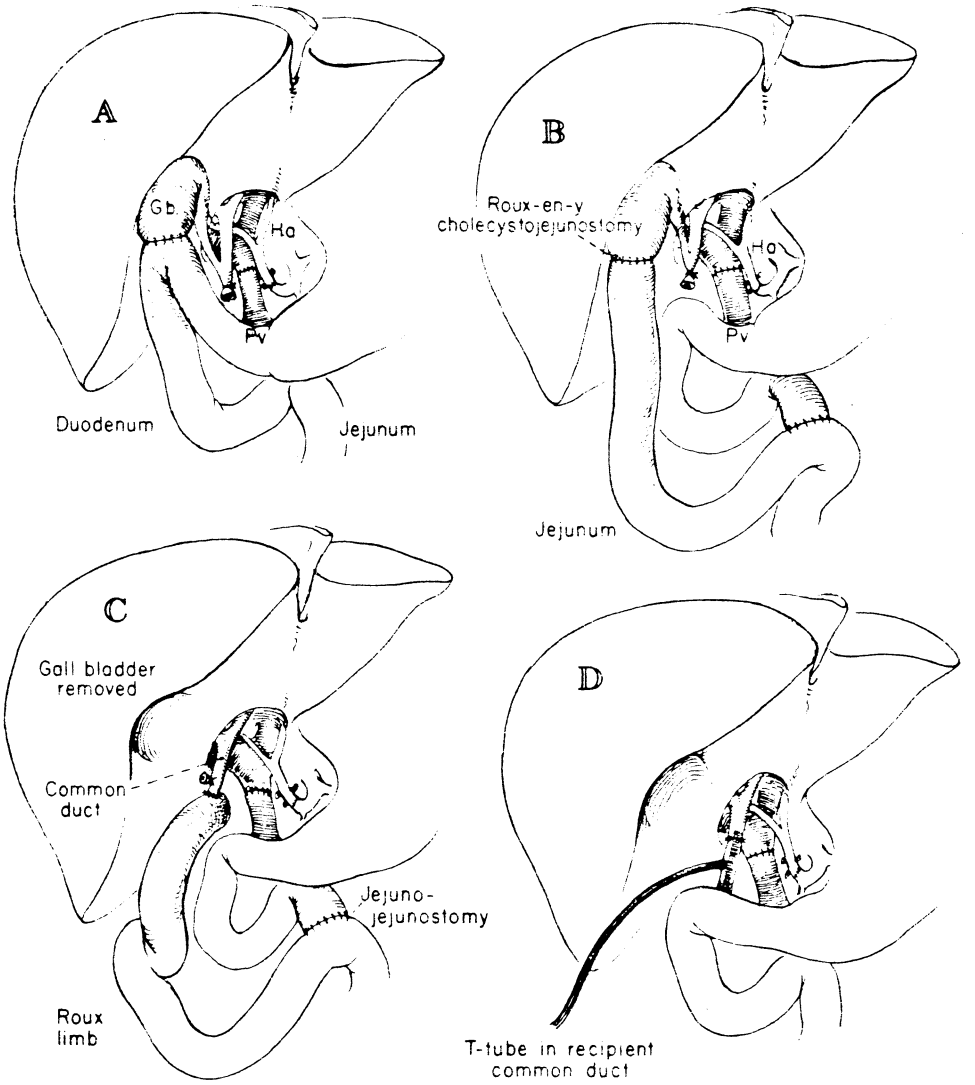


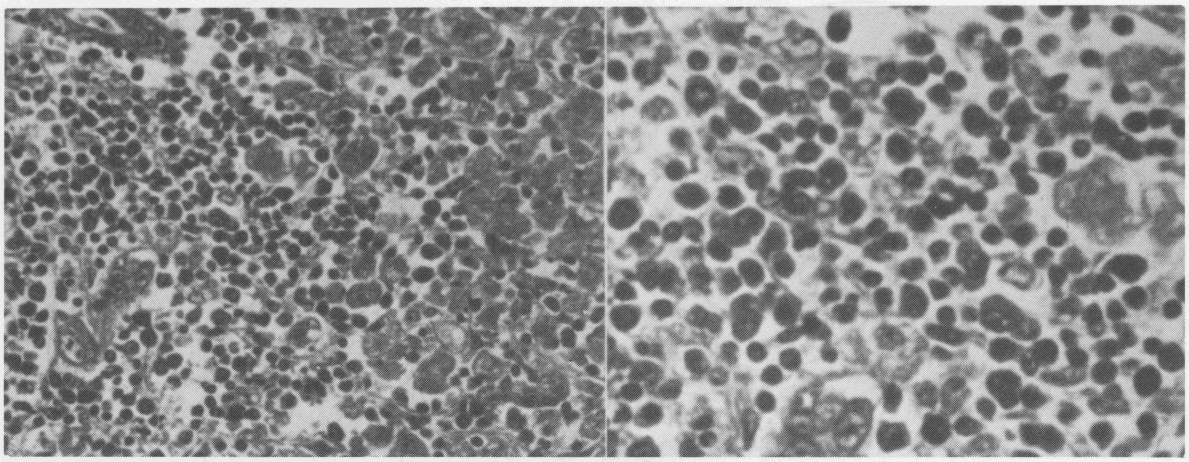
Fig. 3.— Techniques of biliary duct reconstruction used for most of the transplantation recipients. A, Cholecystoduodenostomy. B, Cholecystojejunostomy. C, Choledochojejunostomy after removal of gallbladder. D, Choledochocholedochostomy. Note that the T-tube is placed, if possible, in recipient common duct.

(29, 31). However, in some adults, a safer way has been to transect first the hilar structures and, subsequently, the infrahepatic inferior vena cava when all else is in readiness (Fig. 1). Then, by pulling on clamps which are placed on the hepatic side of these structures, the liver is dissected free from below to above, ligating all cut tissue on the way (Figs. 1 and 2). The suprahepatic inferior vena cava remains intact as the stalk of the specimen until it is clamped just before the liver is removed. The variation in operation has been particularly useful in developing a reasonably long suprahepatic cuff of the inferior vena cava in adults. The vena cava or main hepatic veins may be dissected free from within the cirrhotic liver in a bloodless field (Fig. 2B).

In several of our first recipients who did not have biliary atresia, bile duct reconstruction was with choledochocholedochostomy over a T-tube stent (Fig. 3D). The method lost favor because of a high incidence of bile fistula, and cholecystoduodenostomy after ligation of the common duct (Fig. 3A) became our first choice for a number of years. However, since November 1973, the preferred technique has been cholecystojejunostomy with a Roux-en-Y loop (Fig. 3B),thus removing the homograft from the mainstream of the gastrointestinal tract and draining it through a defunctionalized jejunal limb. Alternatively, Rou-en-Y choledochojejunostomy (Fig. 3C) or choledochocholedochostomy (Fig. 3D) has been used for recently treated patients. In a number of patients, it has been necessary to convert from cholecystojejunostomy to choledochojejunostomy (Fig. 3B and C) because of obstruction at the cystic duct (17).

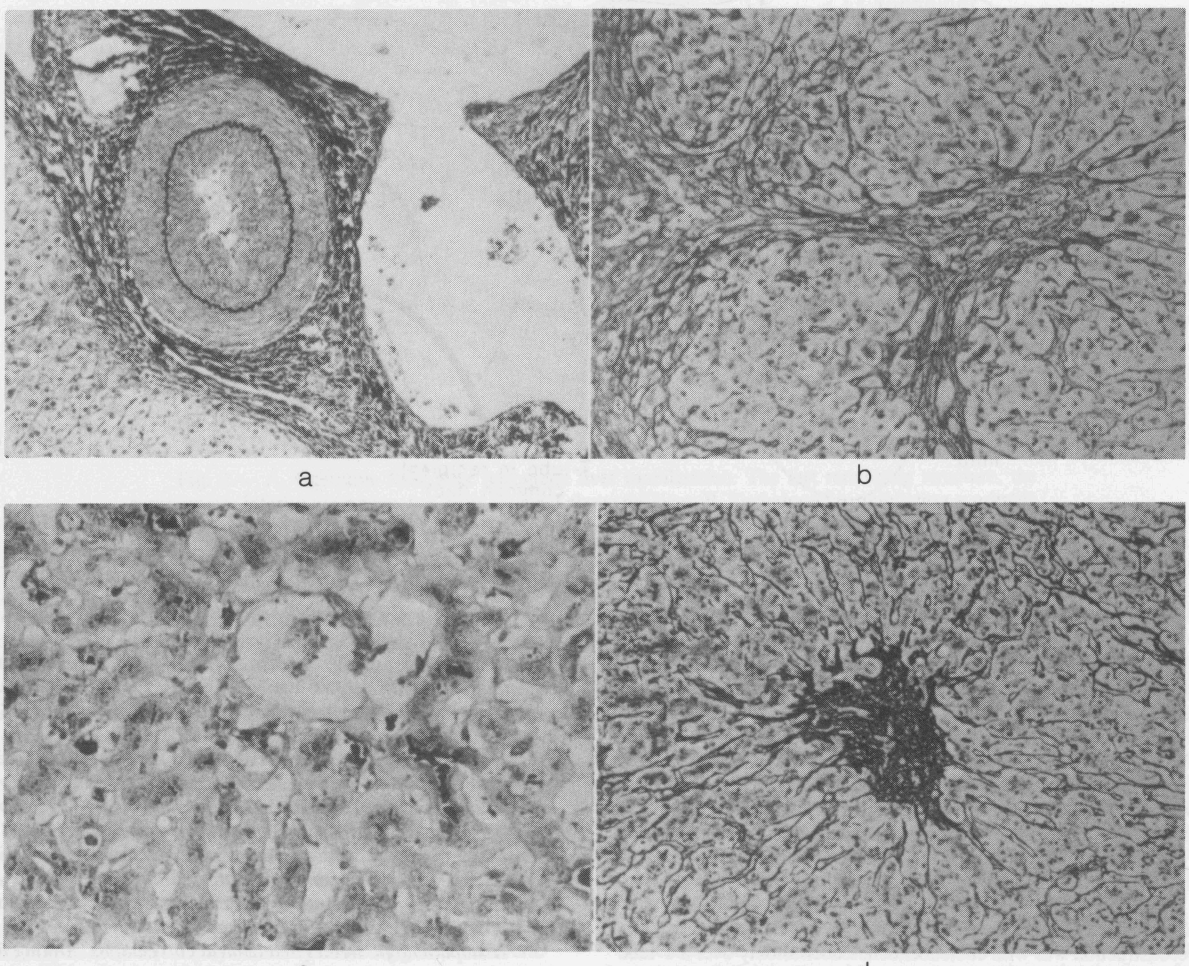
The first five patients were treated with azathioprine and prednisone. The next 88 recipients were given triple drug immunosuppression, which usually consisted of azathioprine and prednisone, to which a two weeks to four months' course of horse antilymphocyte globulin was added (31). If hepatotoxicity of azathioprine was suspected, cyclophosphamide was substituted, since it has an immunosuppressive effect comparable with that of azathioprine (32). In a number of later patients, cyclophosphamide was used at the beginning with a switch later to azathioprine.

Histopathologic studies and clinical correlations. During the 12 1/2 years of study, samples were accumulated from the diseased native livers, homografts and selected other tissues and fixed in 10 per cent buffered Formalin (aqueous solution of formaldehyde). Frozen sections were stained with Sudan IV for fat, and then, the remainder was processed and the paraffin sections were stained with hematoxylin and eosin, Gordon and Sweet's silver impregnation method for reticulin fibers, Perls' Prussian blue method for iron, Weigert's for elastic counter-stained with hematox-



a b

Fig. 4.— Acute rejection. Hepatic homograft, OT 31, nine days after transplantation. Large numbers of lymphoid cells are present in the portal tracts and between the damaged hepatocytes. Hematoxylin and eosin, a, X300; b, X500.



a b c d

Fig. 5.— Chronic rejection. a, A small hepatic artery branch in a graft, OT 16b, 339 days after re-transplantation is almost occluded by intimal thickening. Elastic stain, X60. b, Residual scarring after rejection. Same graft, OT 16b. There is increased portal fibrous tissue and connective tissue septums extend into the lobules. Reticulin, X60. c, Centrilobular cholestasis with bile "thrombi" in dilated canaliculi in graft, OT 14a, one year and 16 days after transplantation. BIle stain, X200. d, Collapse of reticulin fiber framework around central veins in graft, OT 10, 186 days after transplantation. Reticulin, X60.

TABLE III—HL-A TYPING OF PRIMARY GRAFTS IN 93 PATIENTS

*Match**	*No.*	*Survival, one year*	*Alive now*
A	1	1†	0
B	2	1 (50)	0
C	14	3 (21)	2 (14)
D	23	5 (22)	3 (13)
E	40	15 (38)	11 (28)
F	6	1 (17)	0
Not done	7	1 (14)	0
Total	93	27 (29)	16 (17)

Figures in parentheses represent percentages.

*A-match: HL-A identity between recipient and donor; B-match: compatibility between donor and recipient, but fewer antigens determined in the donor; C-match: one antigen incompatible; D-match: two antigens incompatible; E-match: three or four antigens incompatible, and F-match: ABO violation or positive cross match to cytotoxic antibodies.

†Retransplanted after 68 days with C-match graft. Thus, the one year survival was mainly due to the second less well matched organ.

ylin and van Gieson, trichrome for collagen and fibrin, periodic acid-Schiff method for glycogen, Pearse's method for ceroid and lipofuscin, and methyl green pyronin.

Frequently, additional small hepatic samples were initially fixed in glutaraldehyde solution, and then postfixed in osmic acid and embedded in Epon, a synthetic embedding medium. Half micron and ultrathin sections were cut. The former were stained with azure II for examination in the light microscope, while the latter were stained with lead citrate and examined in a Phillips 300 electron microscope.

Many of the findings in the liver grafts have been reported before (21). In preparing this survey, the old specimens were re-examined, and new ones were analyzed. Clinicopathologic correlations were made in each about the reasons for failure. The most important element in the final decision was the state of the homograft and the evidence in it of injury from an old or a new rejection. The clinical course and the findings of the autopsy also were taken into consideration.

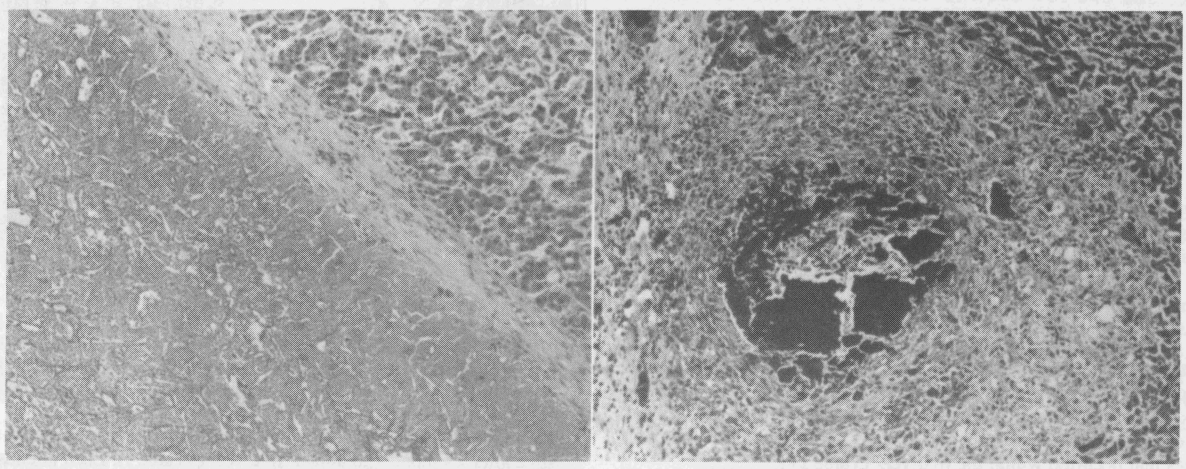
An effort to assign a single, most important cause of failure inevitably required oversimplification, since the terminal events were always complex. Thus, although infection almost invariably played an important role at the end, as has been emphasized before (17, 25, 31), infections are not mentioned hereinafter unless they seemed to play a triggering and primary role. The all important role of infection in these sample patients is to be described in a detailed separate publication.

The effects of hepatic homograft rejection and other postoperative events upon liver function tests have been exhaustively described by us (17, 25, 29, 31) and by Williams and his associates (35). The abnormalities of function with rejection are not diagnostic, since the profile of tests may range from a pattern of obstruction to one of parenchymal necrosis. The single most important test in observing the patients has proved to be serum bilirubin concentration, which frequently will be referred to in the clinicopathologic correlations.

Results

Pediatric patients.

Other than biliary atresia. Nine of the 16 patients lived for at least one year after transplantation. The seven others who received a total of eight grafts died after one to 188 days. Acute rejection (Fig. 4) was directly responsible for one of these seven deaths (Table IV). Late rejection (Fig.



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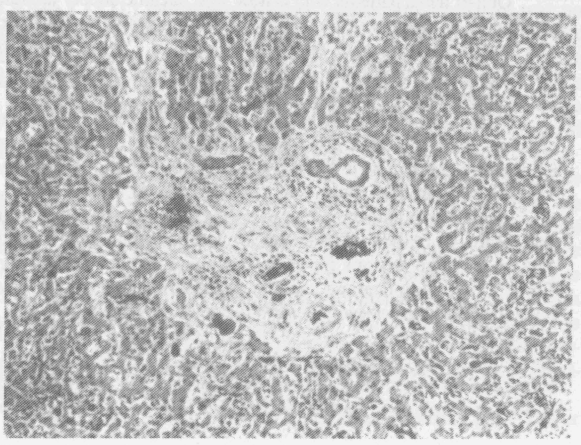
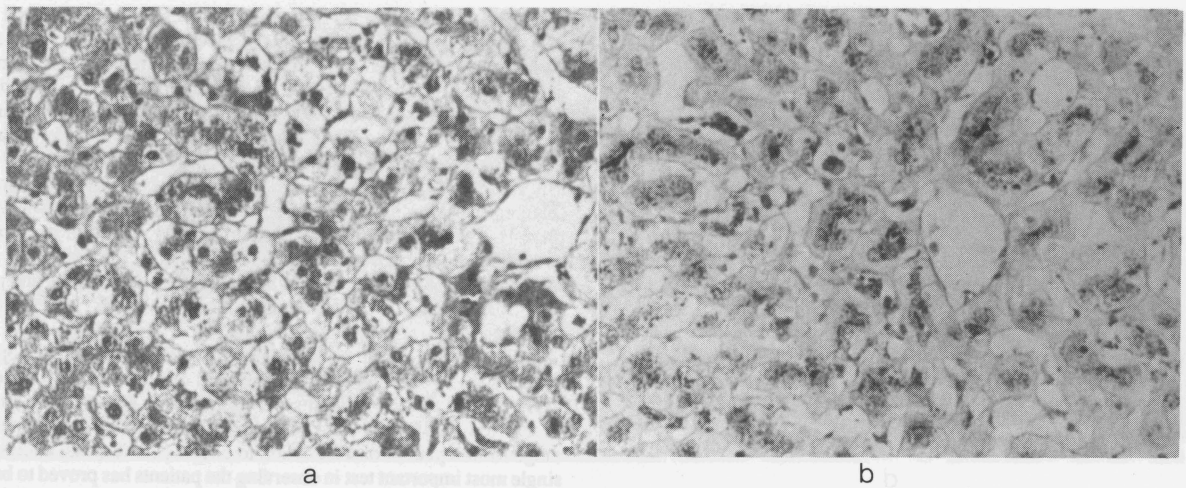


c

Fig. 6.—Large bile duct obstruction. a, Biliary cast syndrome in graft, OT 55, two years and two months after transplantation. The wall of the distended bile duct is fibrous, and the lumen is blocked by inspissated bile and debris. Hematoxylin and eosin, X60. b, Biliary cast syndrome in graft, OT 43, 47 days after transplantation. A portal tract is greatly expanded and an intrahepatic bile duct is blocked by inspissated bile. Hematoxylin and eosin, X50. c, Chronic large duct biliary obstruction. Enlarged fibrous portal tract with proliferated bile ductules containing bile casts. Hematoxylin and eosin, X70.



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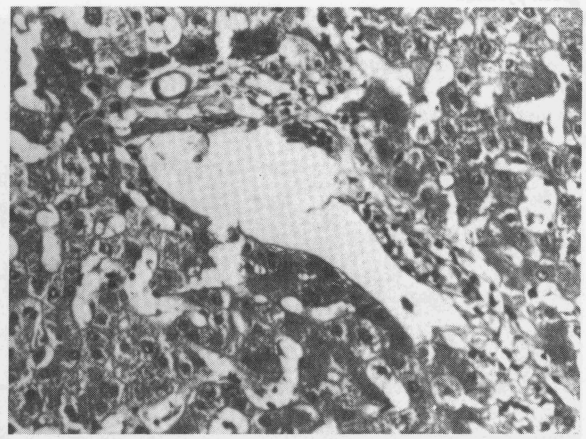

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Fig. 7.— Presumed drug toxcity. Biopsy of a graft in a patient, OT 74, who was deeply jaundiced at one year and five months after transplantation. The liver functions returned to normal after azathioprine was replaced with cyclophosphamide and after the prednisone dosage was lowered to less than half of its previous level. a, Centrilobular hepatocytes are swollen and show abnormal variation in nuclear size. Hematoxylin and eosin, X200. b, Centrilobular cholestasis with bile thrombi in dilated canaliculi. Bile stain, X200. c, Normal portal tract. Hematoxylin and eosin, X200.

5) of a primary graft indirectly led to another death, inasmuch as a retransplantation became necessary and was not successful in spite of the fact that the second organ was not rejected (Table IV). The non-immunologic factors of biliary obstruction, tumor recurrence, perforation of a colonic diverticulum and ischemic liver necrosis were responsible for the other fatalities that occurred within the first year (Table IV).

Shortly after one year (Table IV), two more patients died with recurrences of the hepatomas for which transplantations had been performed. In one of these livers, biliary obstruction developed as a result of a metastasis, and the other one contained evidence of chronic rejection, for which retransplantation was attempted. A third child died 26 months after liver replacement for chronic aggressive hepatitis. At autopsy, a stricture was found at the choledochocholedochostomy site, with dilatation of the duct system which was packed with inspissated bile and debris (Fig. 6). There was no evidence of rejection. The mechanical problem probably could have been corrected if it had been diagnosed. Transhepatic cholangiography had been attempted four times in the six months before death, but the duct system was not entered.

The last late death occurred six years after transplantation. The original diagnosis in this patient was Wilson's disease. After liver replacement, the homograft had no tendency to accumulate copper (30). About four years postoperatively, the patient was thought to have discontinued the immunosuppressive therapy for several months. Liver function, which had been perfect, deteriorated, and he became jaundiced. Resumption of treatment did not reverse the process in spite of the fact that the liver biopsy now had relatively minor abnormalities. Because a transhepatic cholangiogram showed a partial biliary obstruction, bile duct reconstruction was revised with conversion of cholecystoduodenostomy to choledochojejunostomy; jaundice persisted. At autopsy, almost two years later, the main finding in the liver homograft was cholestasis, similar to that seen in Figure 6, suggestive of chronic bile duct obstruction.

Five of the 16 recipients in this nonatresia pediatric subgroup are still alive after 13 months to almost five years (Table I). Each attends junior high school, high school or a university. They all have normal, or nearly normal, liver function, with serum bilirubin concentrations ranging from 0.5 to 1.5 milligrams per cent.

One of these patients who is now well became deeply jaundiced and profoundly ill almost a year and a half after transplantation. A liver biopsy was interpreted as showing drug toxicity (Fig. 7). Cyclophosphamide was substituted for azathioprine with subsequent slow improvement of liver function during the next few months (Fig. 8).

Biliary atresia. The 40 recipients were given a total of 45 livers. One of the second grafts was from a chimpanzee (26, 30). The other four organs for retransplantation were from cadavers as were all the 40 primary grafts. The one year survival figure was 11 of 40 patients or 28 per cent.

In a recent publication, clinical or pathologic analyses were given for each of the 40 individual patients (30). Our interpretations from that raw data of the main reasons for graft losses and deaths are summarized in Table V. The complications came in progressive waves, to which specific etiologic factors selectively contributed at successive times.

Acute problems of surgical technique or management were the most common causes of failure and resulted in the deaths of 11 children in 5.7 ± 6.6 (S.D.) days, range zero to 20. Graft vascular thrombosis, bleeding and the use of necrotic livers headed the list (Table V). The deaths occurred

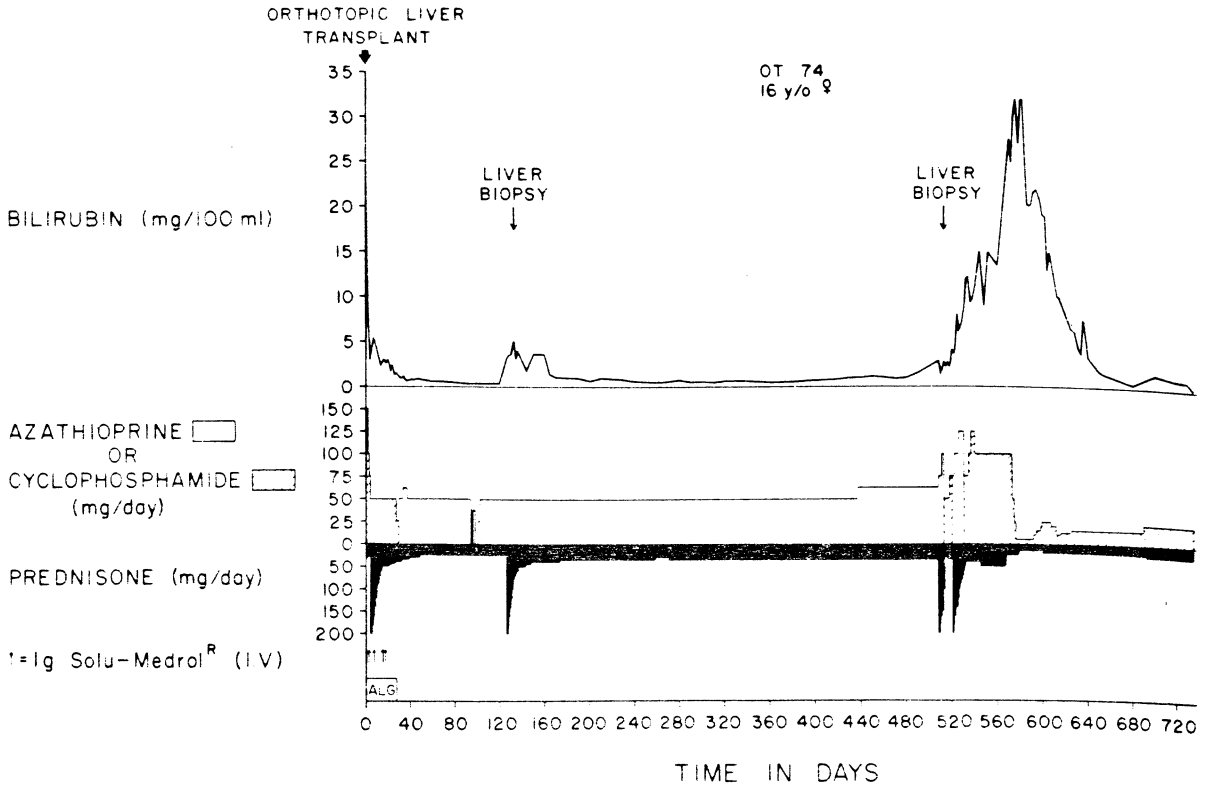


Fig. 8. The first two post-transplantation years of patient, OT 74. The second liver biopsy, which is shown in Figure 7, suggested drug toxicity. Substitution of cyclophosphamide for azathioprine and reductions in steroid dosage were followed by gradual resolution of the profound hyperbilirubinemia.

too soon to permit an effect upon the pre-existing jaundice, except in a child in whom the hepatic artery clotted but who had the longest survival time of 20 days. The serum bilirubin fell promptly and was 1.5 milligrams per cent just before death, which resulted from infection in multiple areas of liver necrosis. Birtch and Moore (4) have reported a similar experience of survival of 45 days by a patient who had a dearterialized and partly necrotic but functioning homograft. The recipient of the chimpanzee heterograft had a serum bilirubin concentration of 14 milligrams per cent after 14 days when he died from pulmonary insufficiency that had been present since the time of retransplantation.

Another seven children died after 60.9±22.9 (S.D.) days after additional mechanical complications which usually involved the reconstructed biliary duct system in one of the ways shown in Figure 9 and which were not immediately lethal (Table V). Infection was the usual final event in the downhill course. The last serum bilirubin concentrations in these patients were 9.9±6.1 (S.D.) milligrams per cent, range 3 to 22. There was no histopathologic evidence of rejection in any of the grafts, nor was cholangitis a prominent feature. Virus infestation of the cells lining the bile ductules or of the hepatocytes was diagnosed in three of these seven livers (Fig. 10). It has been suggested by Martineau and his associates (17) that such virus infestation of the biliary tree can cause swelling, necrosis and shedding of the infected cells to form obstructing casts.

The foregoing pooled 18 examples of predominantly technical or mechanical difficulties accounted for 55 per cent of all the deaths in the atresia experience. The staggering total actually may have understated the true contribution of surgical technical complications to the failures, since it excluded the four examples of septic hepatic infarction (Table V) in which portions of the liver became dearterialized and necrotic with invasion by bacteria from the intestinal tract. The latter complication has been suggested to be due to a combination of insufficient immunosuppression plus technical or mechanical factors which twist or otherwise jeopardize the hepatic arterial patency (25, 31).

Rejection played a surprisingly minor role in causing graft loss or death, at least insofar as this could be detected by histopathologic examination. Four patients acutely rejected the primary graft (Fig. 4) after 17.3±11.0 (S.D.) days. The last serum bilirubin concentrations were 17.8±7.5 (S.D.) milligrams per cent, range 13 to 29.

Three grafts were designated as being lost primarily from chronic rejection because of the severe arterial narrowing, lymphoid cell infiltration and other findings that are characteristic of this process (Fig. 5). These transplants had been in place for two months to two and one-half years and were supporting serum bilirubin concentrations of 19, 10 and 20 miligrams per cent. Three of the four livers that were lost primarily because of septic hepatic infarction had chronic rejection as well.

Systemic infections or else infestations of the homografts were thought to be the main causes of failure in eight patients, generally at an intermediate time. Concomitant chronic rejection had damaged two of the homografts of the five patients who had lethal systemic infection (Table V). Serum bilirubin concentrations were variable in these instances, ranging from 1 to 52, with a mean of 16.0±16.4 (S.D.) milligrams per cent. The three patients with infected livers had bilirubin levels of 52, 15 and 10 milligrams per cent.

One liver which was obtained from an anencephalic monster never cleared bilirubin. Ironically, it had the histopathologic findings of intrahepatic biliary atresia when it was removed 85 days later. An attempt at retransplantation failed when the hepatic artery of the second transplant clotted.

Eleven or 28 per cent of the 40 patients survived for at least one year after operation, and seven are still alive with good or perfect function from their original hepatic homografts after 13, 15, 26, 34, 46, 53 and 71 months (Table I). All are living essentially normal lives. Their return visits to Colorado range from every three to 12 months. None are jaundiced. In two of these patients, serial biopsies were obtained. Some of the early specimens showed classical cell mediated rejection (30) which was readily controlled as judged by the final clinical outcome. As already recounted, the late deaths occurred 12 1/2, 13 1/2, 30 and 41 months after transplant.

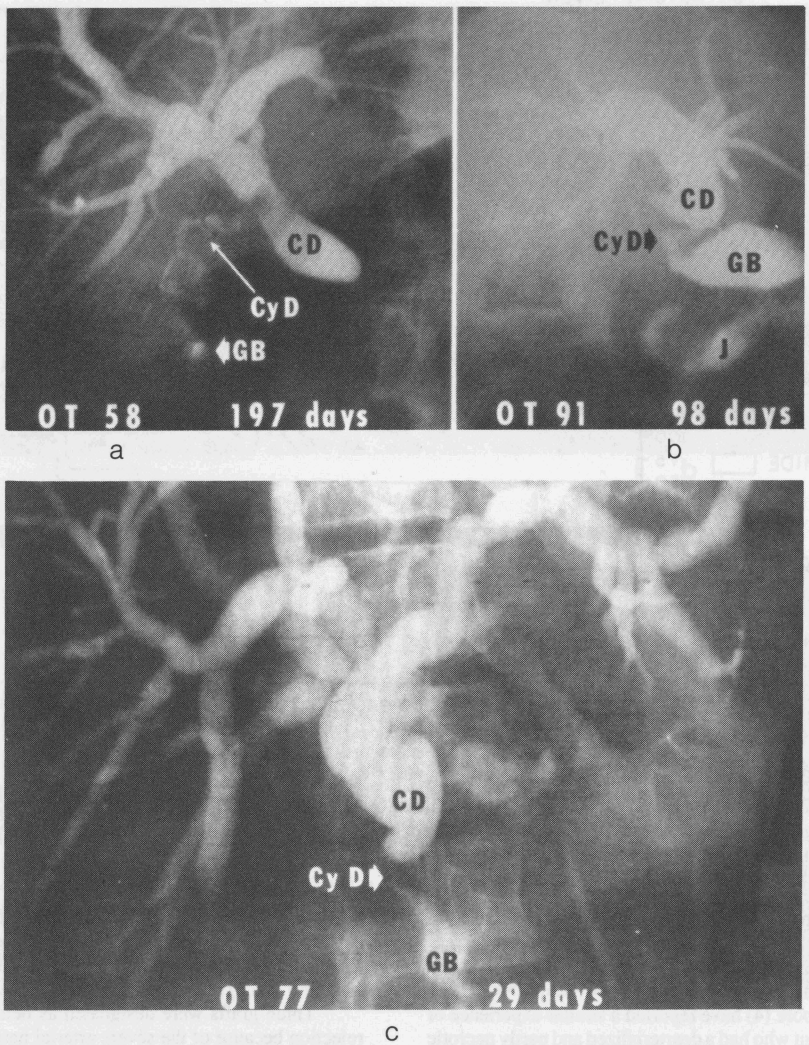


Fig. 9.— Examples revealed by transhepatic cholangiography of homograft cystic duct obstruction after biliary reconstruction with cholecystoenterostomy. a, Original procedure was cholecystoduodenostomy. After this transhepatic cholangiogram, conversion was made to choledochoduodenostomy. At operation, the filling defect near the exit of the cystic duct was found to consist of a chalk-like sludge. There was not complete relief of jaundice. When the patient died 13 months after transplantation, the homograft still had intrahepatic evidence of large duct obstruction. b, The original reconstruction was with cholecysto-Roux-en-Y-jejunostomy. This was converted to a choledochojejunostomy. The patient is well a year later. c, The original reconstruction was with cholecysto-Roux-en-Y-jejunostomy. This was converted to a choledochojejunostomy. The patient is well about two years later. CD, Common bile duct; CyD, cystic duct; GB, gallbladder, and J, Roux-en-Y limb of jejunum.

Adult Patients. Only seven or 19 per cent of the 37 adult recipients lived for as long as one year postoperatively. The results were not satisfactory after transplantation for any of the main indications for liver transplantation (Table II). Only one of nine alcoholics survived for as long as one year. Three of nine patients with chronic aggressive hepatitis lived for this long, as well as two of 12 patients with primary tumors of the liver. Two patients in the malignant category are still alive 15 and 22 months after liver replacement for the small intrahepatic duct cell carcinoma described by Klatskin (15) that obstruct the confluence of the right and left hepatic ducts and cause early jaundice. The recipient with the longer follow-up period has multiple recurrences in the transplanted liver. The patient (Table II) who had the Budd-Chiari syndrome is the only one with this diagnosis known to have been treated by liver transplantation.

In Table VI, a single principal reason was identified for the loss of each of the 35 homografts that were transplanted to the 33 adult recipients who eventually died. Seventeen of the recipients or 59 per cent of the 29 who died in the first postoperative year had lethal complications which had their genesis intraoperatively or just afterward (Table VI).

Eleven of these 17 early accidents were classed as technical and led to death in 19.8 ± 11.4 (S.D.) days. Only one of these 11 patients was not jaundiced before death. The other ten had final serum bilirubin concentrations that ranged from 7 to 46 miligrams per cent, with a mean of 26.8 ± 12.7 (S.D.). Three of the patients were given livers that were irreparably damaged by ischemia in operations that were long and bloody. The grafts of the three more recipients were ruined by tying off an anomalous double barreled channel of cystic and common duct preparatory to performance of cholecystoenterostomy (31). The complication was not recognized or treated in two patients. An attempt to relieve the consequent total biliary

TABLE IV.—CAUSES OF DEATH IN 11 PEDIATRIC PATIENTS WITHOUT BILIARY ATRESIA

OT No.	Time of death, days	Main cause of death	Rejection	Last bilirubin determination, mgm. per cent
20	1	Ischemic liver necrosis	No	—
31	9	Acute liver failure	Yes	30
44	34	Biliary obstruction, unrecognized	No	14
41	61	Biliary obstruction, corrected	No	13
57	64	Perforated diverticulum colon	No	3
23	143	Hepatoma recurrences*	No	40
65	188	Infection after second transplantation at 157 days	Yes healing, first	17
			No, second	1.5
8	400	Hepatoma recurrences;* bile obstruction	No	15
14	436	Hepatoma recurrences;* chronic rejection; complications after second transplantation at 379 days	Yes healing, first	34
			No, second	5
55	780	Biliary obstruction, unrecognized	No	30
27	2190	Chronic liver failure; bile obstruction	Yes healed	16

*Hepatoma was original disease.

obstruction in the third patient resulted in a bile fistula and lethal peritonitis. In five more patients, bile fistulas developed with peritonitis, and they died after 13 to 34 days. The leaks were at the site of choledochocholedochostomy or at the T-tube insertion site in four instances. The fifth fistula was caused by necrosis of the gallbladder after cholecystoenterostomy.

Cerebrovascular accidents accounted for six other intraoperative or puzzling early postoperative complications. Two of the patients were in Stage IV hepatic coma at the time of operation, but the other four were more or less alert. After operation, five or six recipients, including one in previous coma, had a lucid interval that lasted from a few hours to three days before they were crippled by neurologic disabilities that were suggestive of major brain stem damage. The transplant operations in each instance had been long and difficult, with many blood transfusions which further reduced thrombocyte counts that had already been low. The neurologic damage was so profound in each instance that death followed within a few days, or else a decision was later made to stop active treatment. The six patients died after 21.7±14.8 (S.D.) days, range 3 to 41. By then, two patients had serum bilirubin concentrations of 28 and 7 miligrams per cent, respectively, but the other four had bilirubin values which ranged from 0.7 to 2.7 milligrams per cent. All the liver grafts were free of rejection, and two were completely normal. The brains had areas of myelinolysis in the brain stem. In addition, there were other cortical and subcortical abnormalities in some of the brains. These latter included small hemorrhages, infarcts and edema.

There were seven deaths 17.3±9.8 (S.D.) days after transplantation from systemic infections which usually reflected the poor preoperative state of the patients. Two were receiving second grafts, one patient had candidemia at the time of transplantation, one was a fragile end-stage alcoholic and three were patients who were treated early in our experience before infectious disease care under conditions of immunosuppression had been standardized. Two of these last three patients had also had pulmonary embolization from clots which had formed in a venovenous bypass tube which is no longer used (29). The final serum bilirubin concentrations were variable, ranging from 1.5 to 22 milligrams per cent, with a mean of 9.9±7.6 (S.D.).

At an intermediate time of 209±221 (S.D.) days, ranging from 47 to 564 days, six grafts were retrieved by death or retransplantation that had histopathologic findings of extrahepatic biliary obstruction. The original biliary duct reconstruction was with cholecystoduodenostomy or Roux-en-Y cholecystojejunostomy. There was clinical evidence of adequate bile excretion initially. However, it was either proved later in four patients or assumed in retrospect that the site of obstruction was at the cystic duct, as shown in Figure 9. Reoperation was performed in four patients, and a

TABLE V.—REASONS FOR 37 ORTHOTOPIC HOMOGRAFTS AND ONE CHIMPANZEE HETEROGRAFT BEING LOST AFTER TRANSPLANTATION INTO 32 CHILDREN WITH BILIARY ATRESIA WHO EVENTUALLY DIED

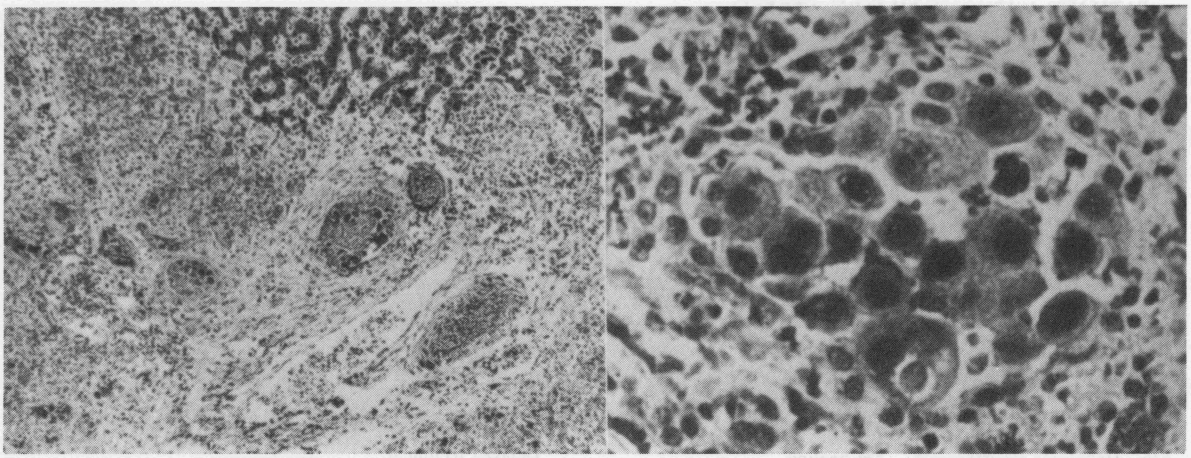
Category	No. of examples	Time to death or graft loss, days	OT No.
Acute technical or management	11		
Hepatic artery clot (3)		4, 20, 1	18, 38, 52b
Hemorrhage (2)		0, 2	1, 76b
Graft necrotic (2)		11, 2	24, 48
Portal vein clot (1)		1	21
Outflow venous obstruction (1)		7	34
Uncorrected hypovolemia (1)		1	80
Pulmonary insufficiency (1)§		14	71b
Delayed technical	7		
Biliary obstruction (4)*		37, 47, 73, 84	30, 43, 49, 84
Biliary fistula (2)*		81, 28	47, 68
Fungus peritonitis and traumatic pancreatitis (1)		76	26
Acute rejection	4	7, 31, 10, 21	7, 50, 71a, 86
Chronic rejection	3	881, 65, 339	13a, 16a, 16b
Septic hepatic infarction	4†	133, 186, 61, 105	9, 10, 11, 12
Infection of graft	3		
Hepatitis (2)		377, 33	29, 76a
Aspergillus (1)		20	13b
Systemic infection	5‡		
? gram-negative bacillus (2)		36, 175	35, 59
Hemophilus (1)		1238	19
Nocardia and Candida (1)		51	37
Herpes (1)		59	67
Transplantation of liver with atresia	1	85	52a

a, Primary graft in patient who underwent retransplantation; b, second graft.
*Bile duct reconstruction was attempted in three of these six patients but unsuccessfully.
†Three of these four livers had vascular or other findings of chronic rejection.
‡Two of these five livers had vascular findings of chronic rejection.
§This child received a chimpanzee heterograft at retransplantation.

secondary choledochoenterostomy was attempted with what was thought to be success. However, histopathologic findings persisted within the liver of large bile duct obstruction weeks or months later. Furthermore, jaundice was not permanently relieved. The final mean serum bilirubin concentration reflecting the function of these grafts was 18.4±8.4 (S.D.) milligrams per cent, range 5 to 28. Two of these apparently obstructed hepatic homografts were removed after 564 and 111 days and replaced under the probably erroneous assumption that they had been rejected. The patients died 11 and 22 days later.

The most surprising observation was that only one of the 35 livers transplanted to adult recipients contained the classical findings of acute cell mediated rejection. Four other grafts contained residual changes of acute rejection or evidence of repair following this process, but rejection was not considered to be the main cause of failure. Furthermore, there were no examples of chronic rejection.

However, only three of the remaining 30 livers were structurally normal. The majority of the others contained such diverse and nonspecific findings (Fig. 11) as fatty infiltration, venous congestion, centrilobular cholestatsis, atrophy or necrosis of the centrilobular hepatocytes and focal necrosis or infarction that were thought to be secondary to ischemia or sepsis. One liver had severe centrilobular atrophy and reticulin collapse at the time of death from hepatic failure after 161 days. A convincing final diagnosis was not made. Several livers were infested with cytomegalovirus



a b

Fig. 10.— Virus infestation of cells lining bile ducts. a, Several bile ducts obstructed by swollen virus infected cells in an expanded portal tract in a liver graft, OT 30, 37 days after transplantation. Hematoxylin and eosin. X50. b, Detail of similar obstructed duct in another infected graft, OT 44, shows enlarged lining epithelial cells containing nuclear inclusions. Cytomegalovirus was identified in this liver. Hematoxylin and eosin, X700.

particles (Fig. 10) which were in hepatocytes and in the cells lining ducts.

Two hepatic grafts were invaded with recurrent malignant tumors of the same kind that had destroyed the native liver, leading to death after 349 and 87 days. One was a hepatoma and the other was a hemangioendothelial sarcoma.

Four of the seven patients who survived for one year are still alive with follow-up periods of 13 to 22 months. They have perfect liver function. All four live at home and work full time or attend school. The patient with the longest follow-up period has recurrences of intrahepatic duct cell carcinoma.

The three deaths after one year were at 13 1/2, 19 1/2, and 20 1/2 months. All were patients whose original diagnosis was chronic aggressive hepatitis. However, recurrence of the aggressive hepatitis was observed only in the recipient who was an HB_sAg virus carrier (Fig. 12). Candidiasis and nocardiosis contributed to her death as Corman and his associates have reported (7). In the other two recipients, the grafts were destroyed by the biliary obstruction already cited, which was not recognized in one instance leading to an ill-advised retransplantation and which was apparently not effectively treated in the other.

Discussion

In a positive sense, the most important conclusion that has emerged from this experience with liver replacement was that prolonged survival repeatedly was possible. A total of 27 patients lived for at least a year following operation, and 16 of this group are still alive after more than one to almost six years. The outlook has slowly improved, although not to a satisfactory state. The first 25 recipients who formed the basis of a monograph on liver transplantation (31) included only five one year survivors. The next group of 25 contained six, and the group after that had eight. There have already been eight one year survivors among the 18 patients beginning with No. 76.

The chronic survivors, particularly those in recent times, have had remarkably stable liver function, and usually, they have achieved complete social rehabilitation. Survival of more than a year after orthotopic liver transplantation has been recorded from other centers by Williams (35), Waldram and Calne and their associates (34) in England, by Daloze and his colleagues (8)[1] in Canada and by Hume and his associates (12) in the United States.

One reason the results were not better was the great technical difficulty of performing orthotopic liver transplantation under the trying conditions of end-stage hepatic disease with consequent portal hypertension and multiple coagulation and metabolic defects. Even excluding the biliary duct complications, about a fourth of the patients who died left the operating room with situations that were incompatible with survival, such as ischemic liver necrosis, graft vascular thrombosis and uncontrolled hemorrhage. The mortality from technical accidents involving vessels was particularly high in infants with biliary atresia, partly because of the small size of the structures for anastomosis. The recent use of microsurgical techniques has almost completely eliminated such losses. In future trials, children with a wide range of diseases, including biliary atresia, undoubtedly will be the most favorable group of recipients.

More critical questions must be asked about the six crippling cerebrovascular accidents which constituted the single most lethal complication intraoperatively, and early postoperatively among the 37 adults. The most consistent, although not the only, damage was found in the brain stem and consisted of the kind of myelinolysis that is associated with end-stage liver disease. Williams and his associates (35) also may have encountered cerebrovascular accidents, inasmuch as two of their 26 recipients failed to awaken from anesthesia. Lampe and his co-workers (16) have reported a serious, but partly reversible, diffuse brain injury in an 11 year old recipient of an orthotopic liver. They ascribed the complication to intraoperative hyperosmolarity that had been caused by excessive glucose administration. Hyperglycemia was not a regular feature of our patients, although it was present in some of them. Intraoperative thrombocytopenia and hypocoagulability may have led to small hemorrhages into areas of preexisting brain disease. Air embolus as the causation has not been ruled out. The neuropathologic findings in these autopsies are being restudied, so that preventive measures may be developed for future patients. In the meanwhile, the high risk of brain damage in adult patients who had a wide variety of diagnoses suggests that as a general policy candidacy for transplantation was being considered too late in the terminal course of the disease.

In the livers that were eventually retrieved for study by death of the recipient or removal at retransplantation, a potentially encouraging but perplexing observation was the paucity of unequivocal findings of either acute or chronic rejection. Early biopsies from a few of those grafts contained the unmistakable lymphoid infiltrations of acute cell mediated rejection (30, 31), but this process usually had been controlled at least by histopathologic criteria by the time the whole organ was examined. Moreover, the kind of chronic rejection that has been so well described in dogs (21, 28) and which is characterized by obliterative vascular lesions with or without lymphoid infiltration and fibrosis was not common either.

About one in four of all the grafts studied had strong histopathologic

1. The patient described in this report is alive one and one-half years after transplantation. The other two one year survivors in the Montreal series of six patients died after two and three-quarters and three and three-quarters years as learned from personal communication on 3 December, 1975.

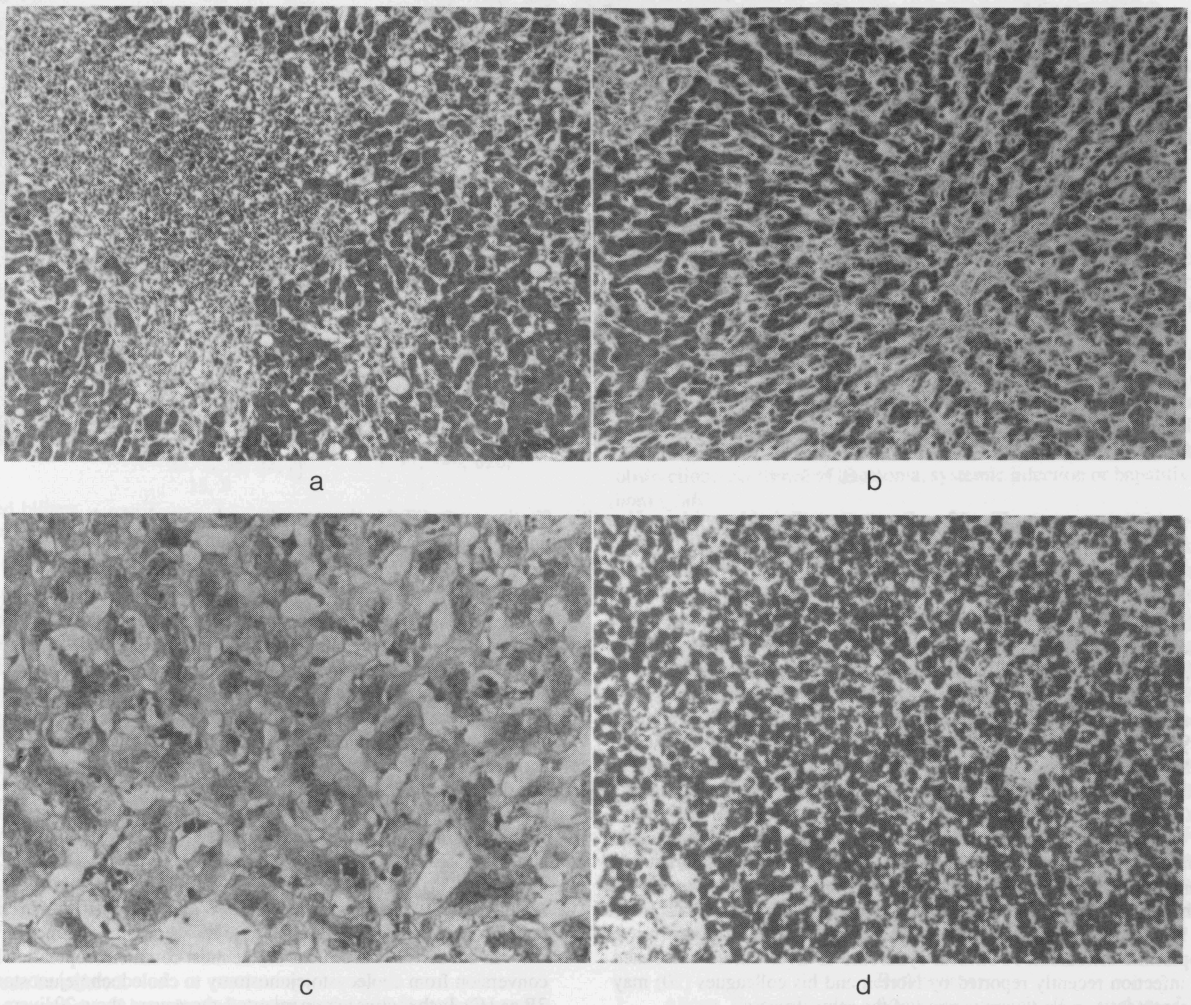


Fig. 11.— Patterns of liver injury not thought to be rejection or obstruction. a, Focal areas of necrosis, OT 69. Hematoxylin and eosin, X60. b, Acute venous congestion, OT 17. Hematoxylin and eosin, X60. c, Centrilobular cholestasis with bile thrombi, OT 97. There was no evidence of large duct obstruction, drug toxicity or rejection in this patient. Hematoxylin and eosin, X200. d, Diffuse fatty infiltration of hepatocytes, OT 94. Frozen section stained with Sudan IV, X60.

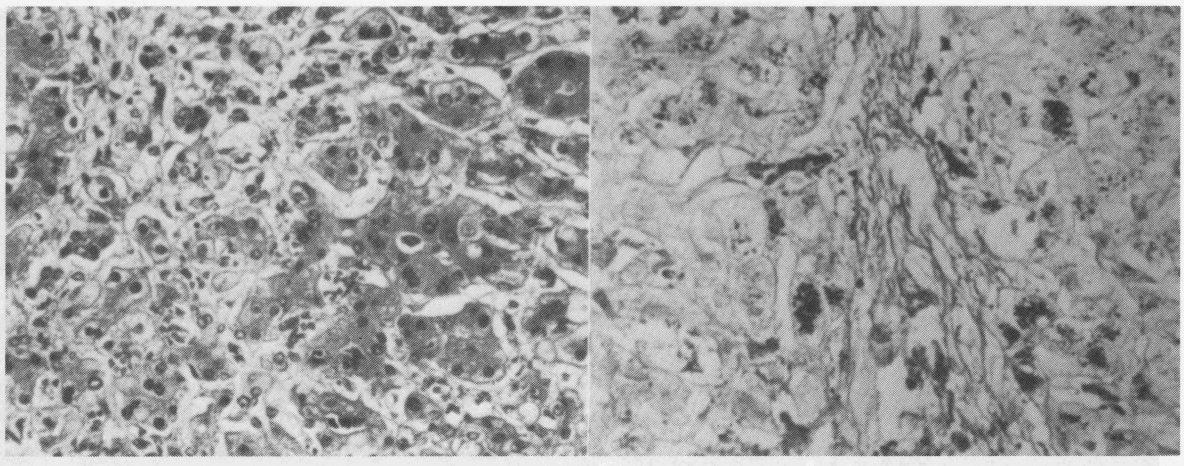
evidence of large bile duct obstruction, and in most of these instances, there was confirmation that such mechanical problems had been present from premortem clinical studies or from postmortem gross examination. Some of the livers had demonstrable infestation and injury by bacteria, fungi or viruses. However, the majority of the livers contained changes that might be considered nonspecific under other circumstances. Examples were fatty infiltration, venous congestion, centrilobular cholestasis, centrilobular atrophy or necrosis and focal necrosis or infarction.

The interpretation from these findings may prove to be that liver recipients are being systematically overimmunosuppressed, particularly since, as a final event, almost all liver recipients who die have significant extrahepatic infections (31). If overtreatment is the problem, a change in management policy will be called for, whereby lightening instead of intensification of immunosuppression would usually be an appropriate response to deterioration of postoperative liver function.

This kind of radical approach can hardly be justified yet since many patients with a graft that is failing early after transplantation respond to increased doses of prednisone. It may be that some of the structural abnormalities that are presently thought to be nonspecific are actually subtle manifestations of hepatic rejection or recovery from this process. Such an interpretation would fit with observations in dogs that start to recover from rejection, either spontaneously or as a result of treatment with immunosuppressive drugs (11, 21, 28, 31). Characteristically, the homografts of such animals lose the lymphoid cell infiltration and develop prominent centrilobular bile stasis, atrophy of the centrilobular hepatocytes, collapse and condensation of the centrilobular reticulin and increased amounts of reticulin and collagen in the portal tracts. Besides any, or all, of these findings, fat deposition is not uncommon.

Similiar observations, including centrilobular bile stasis, have been made in the homografts of untreated, but long surviving, pigs by Hunt (13) and Battersby and his colleagues (2) and in treated baboons by Myburgh and his associates (19). Autografts of dog livers reported by us (31), Alican and Hardy (1) and McBride and his associates (18) did not have the cholestatic or other findings. The same absence of these findings in pig and baboon hepatic autografts has been described by Battersby (2) and Myburgh (19) and their colleagues.

The critical and, as yet, unanswered questions are whether or not the foregoing changes in homografts and particularly the finding of intrahepatic cholestasis could represent ongoing rejection and, if so, how? Myburgh and his associates (19) have suggested the possible dynamic and immunologic nature of the cholestatic lesion on the basis of their own observations as well as a more general hypothesis of Schaffner and Popper (22). In essence, their argument holds that a continuous and often sublethal injury to the hepatocytes is mediated by humoral antibody or by small



a b

Fig. 12.— Recurrence of viral hepatitis. a, Hepatitis affecting graft, OT 36. Hematoxylin and eosin, X250. b, Same graft stained with orcein. Granular material is present in the hepatocyte cytoplasm. This usually indicates the presence of hepatitis B viral antigen. Shikata stain, X300.

numbers of leftover lymphoid cells. The hypothesis continues that hepatocyte organelles, including the smooth endoplasmic reticulum, are damaged, causing perturbations in the secretion and relative concentrations of bile salts, cholesterol, phospholipids and bilirubin with the consequent formation of intrahepatic sludge and the establishment then of a reinforcing cycle of sludging and obstruction. Studies will need to be designed to test this potentially important theory, an extension of which Waldram and his colleagues (34) have applied to partially explain sludge formation in the large bile ducts.

Unfortunately, similar histopathologic abnormalities can be caused by many agents. Fahrländer and his co-workers (9) reported that, in eight patients, severe bacterial infection of organs other than the liver caused intrahepatic cholestasis with minimum hepatocellular injury or inflammation. Electron micrographs revealed dilatation of the canaliculi and rough endoplasmic reticulum. A clinical syndrome of liver failure associated with infection recently reported by Norton and his colleagues (20) may well represent a more severe version of the same process.

It is self evident that intrahepatic cholestasis or the other manifestations of liver injury could also be caused by drugs, particularly those used for immunosuppression. The possibility that either azathioprine or prednisone can harm animal or human livers was already well known a decade ago (27, 31) from experience in kidney transplantation, although there was, at that time, no good way of separating out the instances of serum hepatitis. With the development of the HB_sAg screening tests by Blumberg and his co-authors (5), Torisu and his associates (33) were able to survey the serums of 83 of our renal recipients. Because serial serum samples had been systematically stored, it was possible to develop a longitudinal profile of the HB_sAg state going back as long as five or six years. The serum of nearly 20 per cent of the patients had become HB_sAg positive, but the ultimate incidence of severe liver disease in this group was not substantially different from that in the kidney recipients who had been consistently HB_sAg negative. Because of these observations incriminating factors other than serum hepatitis in the cause of post-transplantation hepatic dysfunction, a number of our renal recipients with serious postoperative liver impairment have been switched to cyclophosphamide (32). The improvement that may follow in the hepatic state without deterioration of renal homograft function has been confirmed by Berne and his associates (3) and in at least one of the liver recipients herein reported. A similar program of drug change in liver recipients with hepatic malfunction is likely to be important in future patients, notwithstanding the belief of Briggs (6) and Ireland (14) and their associates that azathioprine is totally devoid of hepatotoxicity.

Because the definition of the cause for postoperative hepatic malfunction is emerging as the most critical element in the care of liver recipients, other active diagnostic steps will need to be taken, of which repeated needle biopsies and cholangiography are probably the most important. Even if unequivocal diagnosis of conditions like hepatitis, drug hepatotoxicity, rejection and intrahepatic infection is not possible, repeated tissue study findings could give a better insight into the evolution of recovery or failure of the transplants which will be applicable in future patients. Clearly, radical decisions about therapy, such as one for retransplantation, should not be made without prior biopsies and without multiple efforts at transhepatic cholangiography to rule out large duct obstruction.

The greatest dividend from improved diagnosis may be better management of the biliary duct complications that have so beset liver transplantation in our hands (17, 26, 31) and as reported by Williams and his associates (35) in the King's College-Cambridge series in England. Bile fistulas usually require wide drainage. With duct obstruction, reoperation is mandatory before there is incurable infection of the homograft.

In our series of obstructions, the most common reoperation has been conversion from cholecystojejunostomy to choledochojejunostomy (Fig. 3B and C). In the series herein reported, there were about 20 livers in which the ducts were certainly, or probably, obstructed. Only a few were successfully corrected, virtually all within our recent experience, including five of the 16 patients who are still alive.

With such a high incidence of biliary tract complications, continuous reassessment of the initial choice of reconstructive procedure will be necessary. Although our usual first choice is a Roux-en-Y cholecystojejunostomy, choledochocholedochostomy with a T-tube stent has been used frequently, taking pains to provide broad wound drainage. The former operation has had a high incidence of obstruction, and the latter has carried a high risk of fistula (26). With either complication, we (17) and Waldram and his group (34) have noted a remarkable tendency for the ducts to become filled with a chalk-like debris, leading to what has been called the inspissated bile syndrome.

Periodic reassessment also will be necessary of the influence of the original host disease upon the outcome insofar as this factor influences future patient selection. None of the diseases for which liver transplantation has been used so far can be categorically precluded as an indication for further trials, especially in children. The brightest chapter in liver transplantation has been in the treatment of inborn errors of metabolism in children, including our two patients with Wilson's disease, our patient with alpha-1-antitrypsin deficiency and the child of Daloze (8) with Niemann-Pick disease.

Although the experience with adults with cirrhosis in general and with alcoholic cirrhotics in particular has been dismal, this has often been because of their appalling condition at the time of treatment. Earlier decisions need to be made about treatment with transplantation. Even continued efforts to treat recipients with chronic HB_sAg antigenemia are probably warranted, especially if hyperimmune specific gamma globulin

TABLE VI.—PRINCIPAL REASONS FOR 35 ORTHOTOPIC HOMOGRAFTS BEING LOST AFTER TRANSPLANTATION INTO 33 ADULTS WHO EVENTUALLY DIED

Category	*No. of examples*	*Time to death or graft loss, days*	*OT No.*
Acute technical	11		
Graft necrotic (3)		7, 8, 8	6, 75, 85
Iatrogenic duct obstruction (3)*		10, 39, 20	22, 25, 79
Biliary fistula (5)*		23, 13, 27, 29, 34	5, 28, 63, 69, 70
Acute neurologic	6	6, 3, 26, 32, 41, 22	4, 32, 39, 40, 61, 72
Systemic infection§	7	22, 7, 35, 22, 11, 15, 9	2, 3, 17, 54b, 62b, 81, 83
Delayed biliary obstruction	6†	564, 408, 62, 111, 62, 47	54a, 58, 60, 62a, 87, 88
Acute rejection	1‡	9	51
Chronic rejection	0	—	—
Recurrent tumor	2	349, 87	15, 45
Recurrent hepatitis	1	623	36
No satisfactory explanation	1	161	66

a, Primary graft in patient who underwent retransplantation; b, second graft.
*Reoperation was attempted in three of these patients, one with obstruction and two with fistula.
†Attempts were made to operate secondarily upon four of these patients.
‡Four other grafts contained residual changes or repair following acute rejection, but this was not considered to be the main cause of failure.
§Bacterial or fungal or both.

therapy can be offered.

The treatment of hepatic malignant tumors with liver replacement remains a controversial issue. One of our patients is cured of a hepatoma after almost six years, but that neoplasm was small and was an incidental finding in a liver that had biliary atresia. Five other patients with hepatomas who lived long enough for observations to be made had recurrences as did a sixth patient whose native liver and, later, whose liver graft were destroyed by a hemangioendothelial sarcoma. Two of our three patients with intrahepatic duct cell carcinomas are alive one and one-quarter and one and three-quarter years postoperatively. Unfortunately, the patient with the long survival period has massive recurrences which are predominantly in the homograft.

Although details have varied, the over-all message from Williams and his associates (35) has been the same, although somewhat more optimistically expressed. In their series of transplantations for four hepatomas, five duct cell carcinomas, two metastatic malignant lesions and one cholangiocarcinoma, eight of the 12 patients were afflicted with recurrent neoplasm. In four of their five patients with duct cell carcinoma, obvious metastases developed, the exception being a recipient who died after three weeks. On the other hand, hepatoma recurred once in four instances, but only one of these recipients lived as long as one year. That exceptional patient who died of biliary obstruction after more than five years was free of metastases at autopsy. However, the original tumor was apparently slow growing, since she had been aware of an abdominal mass for more than six years preceding transplantation.

Recurrences after liver transplantation for primary hepatic malignant tumors also have been recorded by Hume (12) and Fortner (10) and their associates. It is axiomatic that the outcome in any given patient will depend on the extent of the neoplasm at the time of transplantation. In this connection, a precautionary note about what constitutes genuine candidacy for liver replacement may be introduced on the basis of our unusually large experience with hepatic trisegmentectomy, a partial liver resection in which approximately 85 per cent of the liver is removed (24). A number of the patients in that series who, as it turned out, could be treated by conventional means had been referred to us for consideration of liver transplantation after an erroneous decision of nonresectability had been made at earlier operations. The same point has been made by Smith (23).

Summary

During the 11 1/2 year period ending 13 months ago, 93 consecutive patients were treated with orthotopic liver transplantation. Fifty-six of the recipients were 18 years old or younger, and the other 37 were adults. The most common indications for operation were biliary atresia, primary hepatic malignant tumor, chronic aggressive hepatitis and alcoholic cirrhosis.

There has been a gradual improvement in results throughout the period of study, although not to a satisfactory level. Twenty-seven of the 93 patients survived for at least one year after liver replacement with a maximum of six years, and 16 are still alive after 13 to 71 months. The 11 late deaths after one to six years were caused by chronic rejection, biliary obstruction, recurrence of hepatoma, systemic infection or hepatitis of the homograft.

Rejection of the liver as judged by classical histopathologic criteria played a surprisingly small role in the heavy over-all mortality, accounting for less that 10 per cent of the deaths. Technical or mechanical problems, especially those of biliary duct reconstruction, were a far greater cause of failure, as were systemic infections. Six of the 37 adult recipients had lethal cerebrovascular accidents during, or just after, operation. When abnormalities of liver function developed in the postoperative period, the nearly automatic diagnosis of homograft rejection, in retrospect, proved to have been wrong in most instances.

Further development of liver transplantation depends upon two kinds of progress. There must be reduction of operative and early postoperative accidents and complications by more discriminating patient selection, purely technical improvement and better standardization of biliary duct reconstruction. The second area will be in sharpening the criteria for the differential diagnosis of postoperative hepatic malfunction, including the liberal use of transhepatic cholangiography and needle biopsy. Only then can better decisions be made about changes in medication or about the need for secondary corrective surgical procedures.

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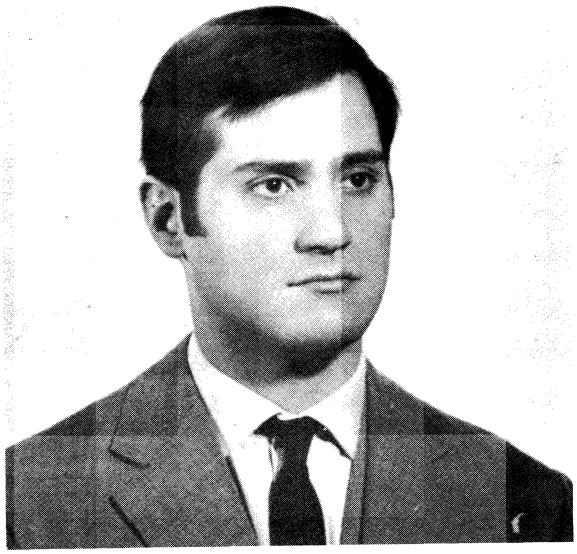

J. C. Cutropia

Passive veno-venous bypasses were described in Part I. In 1972, Cutropia of Mendoza, Argentina published the first description of pump-driven veno-venous bypass without heparin. Dr. Cutropia was then 31 years old. He is now Chief of Surgery and Director at Hospital Español of Mendoza, Argentina. Dr. Cutropia's work was not known at the time a similar technique was applied clinically in Pittsburgh.

Transplante hepático ortotópico experimental
Experimental orthotopic liver transplantation

Revista exspañola de las enfermedades del aparato digestivo, 38: 553-70, 1972

J. C. Cutropia, F. Coratolo, A. Spinetta, J. Kei, A. Ribas, L. Assini, G. Delle Donne, M. Bianchi, A. Guiñazu and J. A. Verdaguer

The study was conducted at the Department of Surgical Research of the Third Chair of Clinical Surgery, Mendoza University School of Medicine and the Español Hospital of Mendoza, Argentina.

Orthotopic liver transplantation was carried out in the following stages: harvesting of the donor liver, recipient hepatectomy without interruption of the posterior vena cava and the orthotopic placement of the donor liver.

Prior to this series, 38 experimental transplantations were conducted in order to perfect the surgical technique as well as postoperative management.

Materials and methods.

This series was comprised of 40 healthy mongrel dogs of either sex with weights ranging from 15 to 27 kg; 20 animals were donors and an equal number were recipients.

Prior to surgery they were given liquids exclusively.

No immunosuppression was administered.

Anesthesia. Anesthetic agents were adminsitered by venotomy in the donors and by cephalic vein cutdown in the recipients. Respiration with oxygen was maintained by mechanical ventilation. The induction of anesthesia was achieved with droperidol-fentanyl, 2cc/10 kg, diacepinone chlorobenzene, 1mg/kg; the second stage of anesthesia was sustained with sodium thiopental, 5mg/kg. and galamine, 2 mg/kg.

Reversal was obtained with atropine sulfate, 0.5 mg/10 kg, niketamine, 0.15 mg/kg and neostigmine, 0.5 mg/10 kg.

Surgical technique regarding the donor.

— Midline incision from the xyphoid to the pubis.
— Division of falciform ligament, triangular ligaments and of the gastrohepatic omentum.
— Dissection of the common hepatic artery as far as the celiac axis, ligation and division of the pyloric and gastroduodenal arteries.
— Identification and division of the common bile duct below the confluence with the cystic duct.
— Dissection of the portal vein as far as the gastrosplenic vein, ligation and division of the pyloric and gastroduodenal veins.
— Dissection of the posterior vena cava from the diaphragm as far as the right supradrenal vein.
— Division of the suprahepatic posterior vena cava by means of a pericaval phrenicotomy.

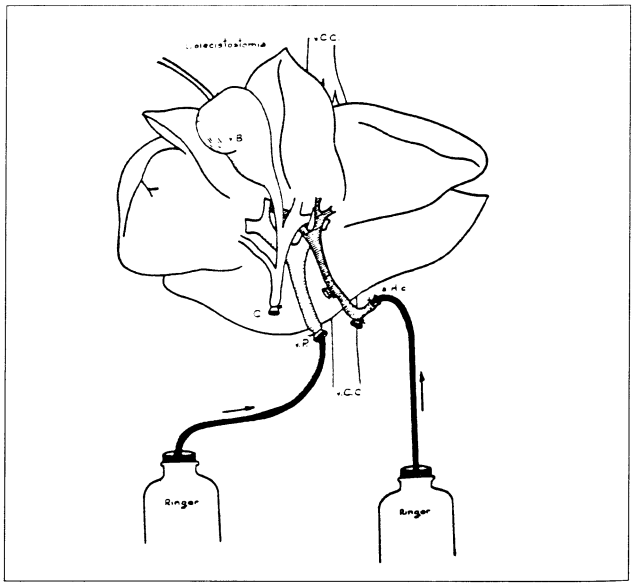

Fig. 1.— Gravity perfusion of the donor liver. v.C.C., caudate vena cava; a.H.c., common hepatic artery; v.P., portal vein; c. common bile duct; v.B., gallbladder.

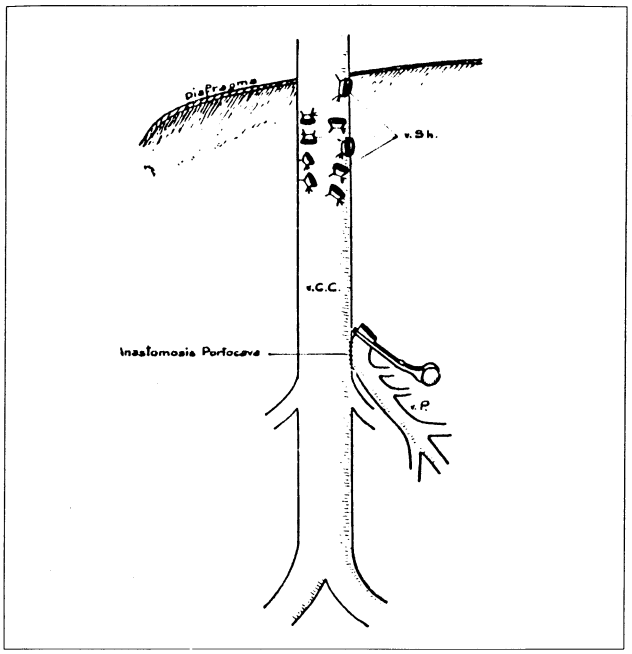

Fig. 2.— Recipient hepatectomy sparing the caudate vena cava. v.Sh., suprahepatic veins; v.C.C., caudate vena cava; v.P., portal vein.

— Division of the common hepatic artery in continuity with the celiac axis with the introduction of a thin catheter permitting perfusion.
— Division of the portal vein with the introduction of a catheter permitting perfusion.
— Puncture of the base of the gallbladder with lavage and the creation of a cholecystostomy with a Pezzer tube.

The perfused organ (Fig. 1) is carried to the operating table on a tray. For perfusion at 4-8°C, 500 cc of lactated Ringer's solution was mixed with procvaine, 1 g, heparin, 5000 units, protease inhibitor, 100,000 units, sodium bicarbonate, 40 meq.

Perfusion was accomplsihed by gravity with a 140 cm column for the arterial line and a 30 cm cloumn for the venous line. The ratio between flow

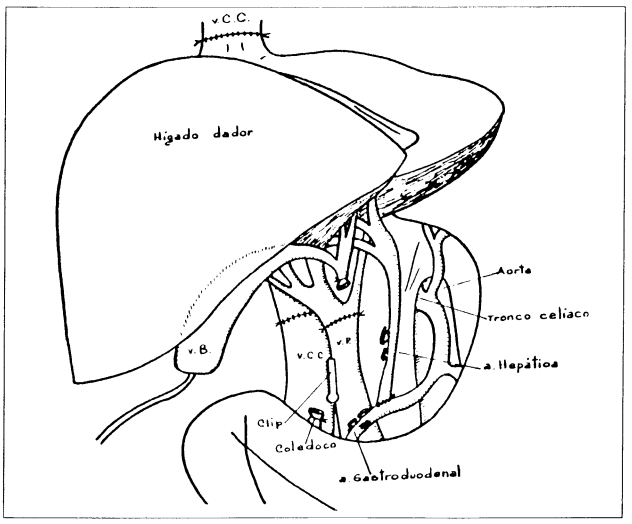

Fig. 4.— Vascular reconstruction in the recipient. The portacaval shunt is occluded with a clip. v.C.C., caudate vena cava; v.P., portal vein; v.B., gallbladder.

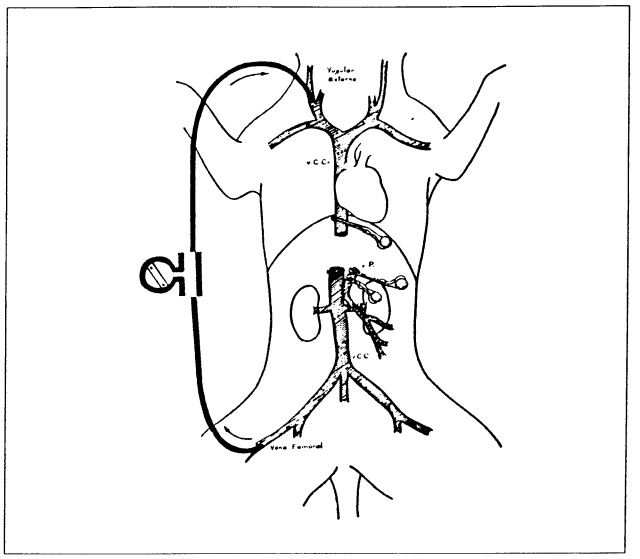

Fig. 3.— Decompression of the caudate vena cava and the portal vein in the recipient while these vessels is accomplished by means of a roller pump or a simple tube. v.C.C., craniad vena cava; v.P., portal vein; v.C.C., caudate vena cava.

to the hepatic artery and portal vein was maintained at 1:2, i.e., 1000 cc perfused through the arterial side for 500 cc perfused through the venous side.

The donor was exsanguinated by means of aortic puncture. In this way 200-400 cc of blood was obtained.

Operative technique regarding the recipient.

— Midline incision from the xyphoid to the pubis.
— Division of the falciform ligament, the triangular ligaments and the gastrohepatic omentum with meticulous hemostasis.
— Visualization of the common hepatic artery through the gastrohepatic space. The hepatic arteries are identified and dissected.
— Identification of the common bile duct proximal to the duodenum.
— Dissection of the portal vein from the hilum of the liver as far as the duodenal vein.
— Dissection of the infrahepatic vena cava as far as the renal veins.
— Wide side-to-side portacaval anastomosis* with 5-0 silicone-treated silk with a continuous everting suture.
— Ligation and division of the hepatic arteries.
— Ligation and division of the common bile duct.
— Division of the portal vein between vascular clamps proximal to the hepatic hilum.
— Placement of two sutures in the diaphragm on either side of the vena cava; these are used to pull the liver and diaphragm caudally.
— Individual dissection and division of all hepatic veins; thus, an anhepatic animal is obtained and the posterior vena cava remains intact (Fig. 2).
— Construction of an external right femoro-jugular bypass with silicone-treated plastic tubes. The tubes are silicone-treated for 30 minutes with water-soluble silicone diluted 1:20 in distilled water and then sterilized in an autoclave.
In the first ten animals a roller perfusion pump was used for veno-venous bypass; in the remaining ten animals the bypass was passive with a simple tube (Fig. 3).
— Division of the posterior vena cava between vascular clamps leaving a venous cuff of sufficient length to permit anastomosis.
— End-to-end anastomosis of the recipient's subdiaphragmatic vena cava with the donor liver's suprahepatic vena cava using 5-0 silicone-

*Actually mesocaval.

treated silk in a continuous everting suture.
- End-to-end anastomosis of the donor liver's infrahepatic vena cava with the recipient's suprarenal vena cava with the same material and in the same manner as in the previous anastomosis.
- Removal of the vascular clamps from the posterior vena cava and the retention sutures from the diaphragm. After placing the liver properly in the right subdiaphragmatic space, the pericaval diaphragmatic cuff is fixed to the muscle of the right hemidiaphragm.
- Retrograde drainage of as much as 100 cc of blood from the donor's portal vein. End-to-end anastomosis of the donor portal vein with the recipients portal vein with 5-0 silicone-treated silk in a continuous everting suture. The vascular clamps are removed.
- End-to-side anastomosis of the donor's common hepatic artery to the arch of the recipient's common hepatic artery with 6-0 silicone-treated silk.
- Closure of the portacaval anastomosis with a plastic clip(Fig. 4).
- Exteriorization of the cholecystostomy drain.
- Closure in individual layers of the abdominal wound.
- Removal of the shunt tubes.

Intraoperative care of the recipient.

During hepatectomy 500 cc of lactated Ringer's solution was administered, and from the onset of the anhepatic state to completion of the procedure 500 cc of lactated Ringer's solution with glucose, 0.5 g/kg/hr, and sodium bicarbonate, 2 meq/kg/hr, was administered. At the conclusion of the vascular anastomoses, 500 cc of blood 100,000 units of proteinase inhibitor and 4 g of E.A.C.A. (epsilon-aminocaproic acid) was infused.

For every unit of blood infused (300 cc) sodium bicarbonate, 10 meq., and calcium gluconate , 200 mg, are injected.

Also, during each hour of the procedure the recipient was administered 50 cc of 15% mannitol and the diuretic response was observed.

Postoperative care of the recipient.

Cannulation of the vein was maintained for 72 hours and 50 cc/kg/hr of 5% glucose in physiological saline was infused. Every eight hours chloramphenicol, 1 g, and analgesics were adminstered by venoclysis. After the third day this was done intramuscularly. Oral alimentation, first liquids and then solids, was initiated as soon as possible.

Studies in recipients.
- In all animals the biliary secretion was measured every 24 hours. -Total bilirubin and the direct and indirect fractions, SGPT and alkaline phosphatase were determined daily in all recipents by spectrophotocolorimetry.
- Angiography, fluoroscopically and with images, was performed using sodium diatrizoate. During the immediate postoperative period in recipient 13 selective arteriography of the liver was performed via the left femoral artery. On the second day, in recipient 14, venography of the vena cava by means of the left femoral vein and, in recipent 15, splenoportography by pubcture of the spleen were performed.
- Cholecystography with sodium diatrizoate venography of the vena cava in recipient 14 on the same day.
- Autopsy in all animals.
- Microscopic examination of all transplanted livers after fixation in 10% formol with paraffin and staining with hematoxylin-eosin.

Results.

The average length of the operation was 270 minutes.

The average time of hepatic perfusion was 20 minutes, the average time for completion of the hepatectomy was 170 minutes and the average time for the vascular anastomoses was 80 minutes.

The shortest survival (Fig. 5) was a few minutes and the longest was six days.

Clinical observations.

A short time after the cessation of respiratory assistance in recipient 3 the animal became cyanotic and died without ever resuming adequate spontaneous respiration.

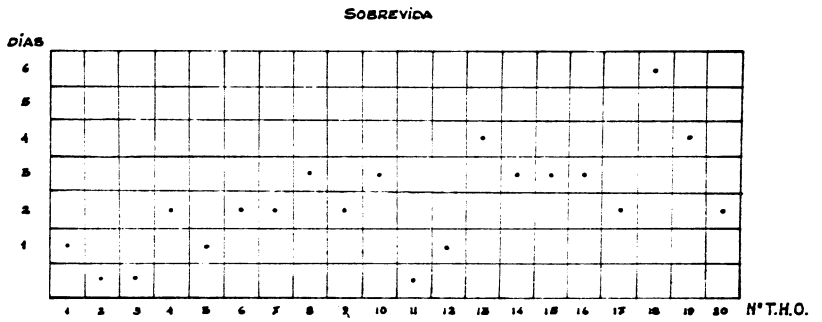

Fig. 5.— Survival of 20 orthotopic liver transplants.

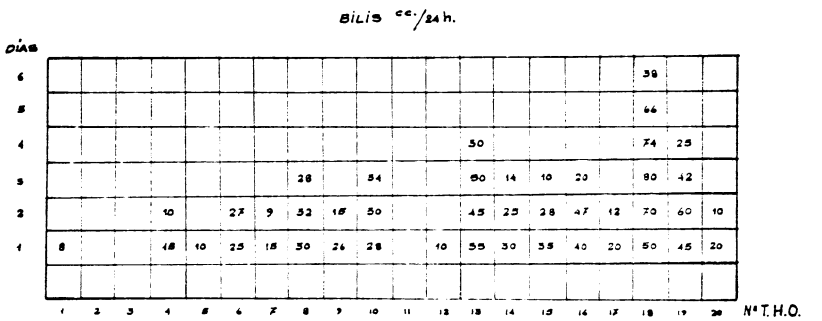

Fig. 6.— Bile drainage via cholecystostomy in 20 orthotopic liver transplants.

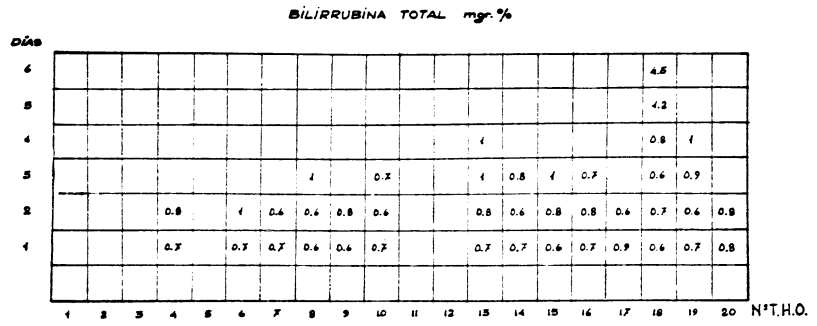

Fig. 7.— Total serum bilirubin determined in 20 animals with orthotopic livers transplants.

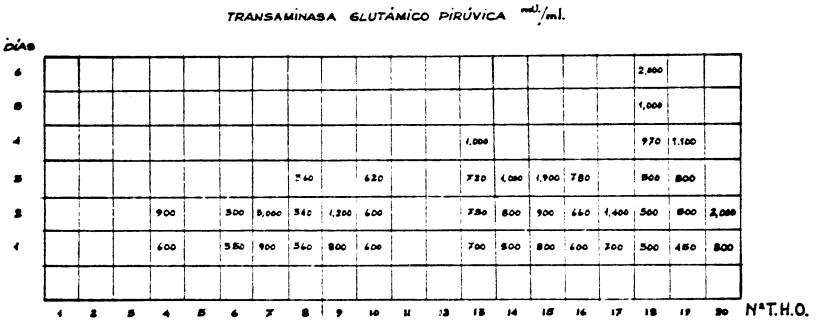

Fig. 8. —Glutamine pyruvate transaminase levels in 20 dogs with orthotopic liver transplants.

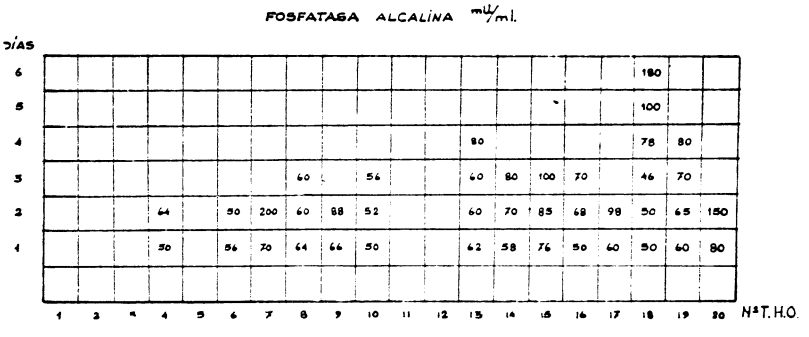

Fig. 9.— Alkaline phosphatase levels in 20 homogeneic orthotopic liver transplants.

Recipents 2 and 11 died 20 and 10 minutes after the operation, respectively.

Recipients 1, 5, 12 and 16 bled copiously from their laparotomy wounds.

After recovering from anesthesia, recipients 1, 4, 5, 7 and 12 had dyspnea, pallor of the mucosa and conjunctiva, cyanosis and some vomiting that continued until the dogs died.

Recipients 13, 16 and 19 had unremarkable postoperative courses until the third day; then they became tremulous, vomited, had convulsions and died.

On the sixth postoperative day after emesese, anorexia, weakness and prostration, recipient 6 became unconscious, icteric and bilirubinuric.

After unremarkable postoperative courses and adequate voluntary oral feedings, recipients 8 and 10 died without any obvious signs or symptoms.

Laboratory results.

During the days before the death of recipent 18, the majority of the total bilirubin was comprised of the conjugated fraction.

Immediately following the operation, the values of SGPT (Fig. 8) and alkaline phosphatase were elevated, and they attained their highest levels in recipients 7, 18 and 20.

Radiologic findings.

Selective arteriography of recipient 13 demonstrated (Fig. 10) that the anastomisis was intact and perfusion of the liver was good.

Venography of the cava in recipient 14 (Fig. 11) revealed a stenosis at the level of the infra- and suprahepatic posterior vena cavae caused by the unequal diameters of the two vessels with retrograde filling of the suprahepatic vena cava.

Splenoportography in recipient 15 (Fig. 12) demonstrated an obstruction to flow at the portal vein anastomosis casued by stenosis and thrombosis.

Cholecystocholangiography (Fig. 13) in recipient 14 demonstrated the point of ligation and division of the common bile duct as well as the intrahepatic flow of bile.

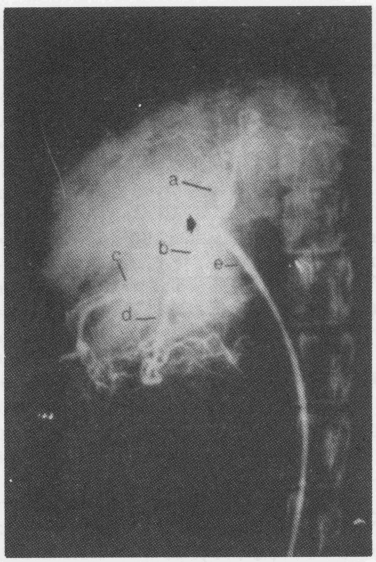

Fig. 10. — Selective hepatic arteriography in recipient 13. The arrow indicated the anastomosis of the donor and recipient hepatic arteries. *a,* donor common hepatic artery and hepatic artery; *b,* recipient gastroduodenal aretry; *c,* craniad portion of recipient pancreaticoduodenal artery; *d,* recipient right gastroepiploic artery; *e,* recipient common hepatic artery.

Autopsy findings.

In recipients 1, 2, 5, 11 and 12 autopsy revealed 200-700 cc of blood in the abdominal cavity, and in the remaining animals there was 50-600 cc of anthochromic fluid.

In all of the animals the liver was tense and congested.

In recipients 4, 6, 7, 9, 17 and 20 the gallbladders contained 18-22 cc of bile; in the remaining animals there was 5-10 cc.

Recipients 18 and 19 had hepatic abscesses.

Rupture of the hepatic parenchyma was observed in recipients 1, 5 and 12.

Anastomoses involving the portal vein were narrowed in recipients 4, 5, 7, 15 and 17, involving the subdiaphragmatic vena cava in recipients 8, 14 and 17 and involving arteries in recipients 4, 6, 7, 12 and 20.

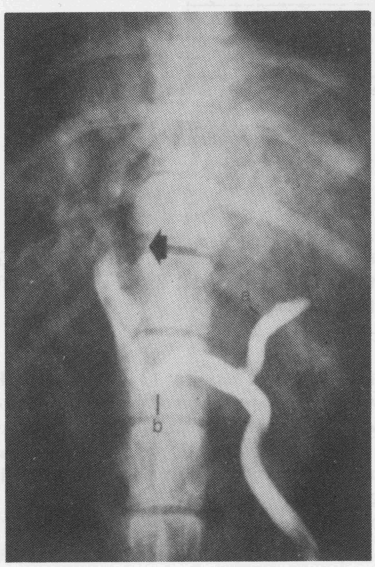

Fig. 12. — Splenoportography of recipient 15. The arrow demonstrates the narrowed anastomosis between the donor and recipient portal veins. *a,* dilated recipient left gastric vein; *b,* dilated recipient portal vein.

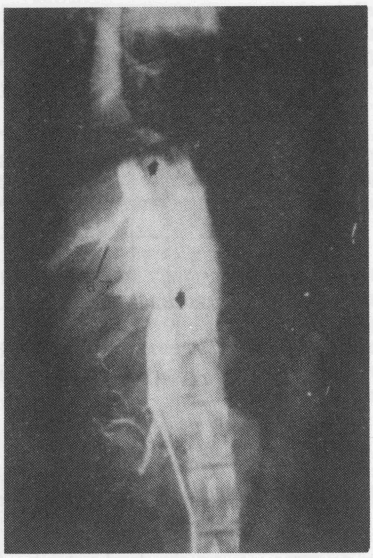

Fig. 11. — Venacavography of recipient 14. The arrows indicate the narrowed anastomosis of the donor and recipient vena cavae. *a,* suprahepatic veins that are visualized because of the poor drainage of the posterior vena cava.

In recipients 2 and 11 the suprahepatic cavocaval anastomoses were intact dorsally.

Thrombosis of the portal anastomosis was noted in recipient 15 and in the arterial anastomoses in recipients 7, 12, and 20.

Splanchnic venous congestion was observed in recipients 4, 7, 9, 14, 15 and 17, and in recipients 9, 14, 15 and 17 there was a large amount of blood in the intestines.

Recipient 6 had massive atelectasis of the right lung and in recipient 10 the lungs were markedly congested.

In recipient 6 there was an extensive area of necrosis of the epicardium of the left ventricle.

Microscopic findings in transplanted livers.

— Congestion with sinusoidal distension and rupture of the centrilobular veins as seen in the classic blue livers in recipients 1, 4, 5, 6, 7, 8, 9, 10, 14 and 17 (Fig. 14).

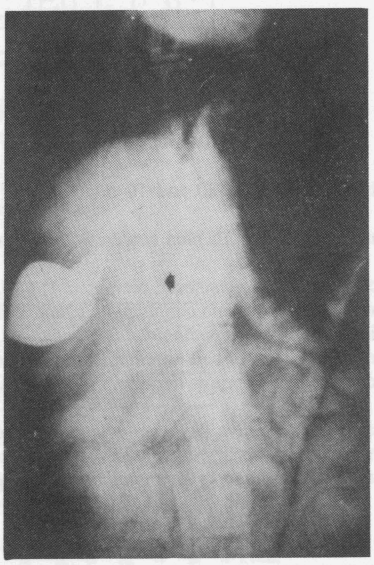

Fig. 13. — Cholecystangiography. The arrow indicates the point where the donor common bile duct is ligated and divided.

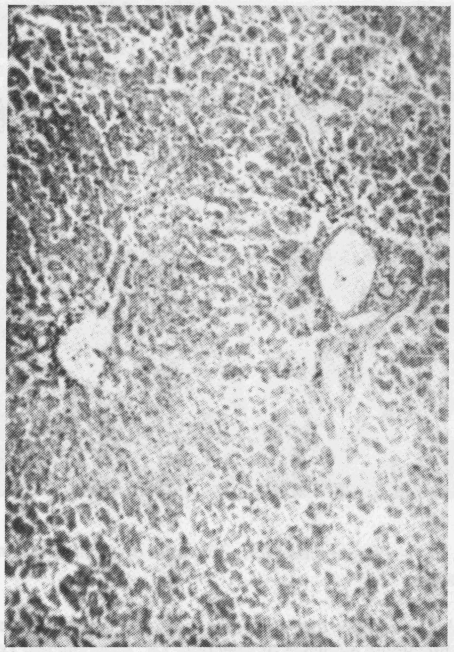

Fig. 14. — Photomicrograph, 42. Areas of collapse and distension of the sinusoids. Rupture of the central vein of the lobule. Diffuse mononuclear cell infiltration.

- Congestion with moderate infiltration of lymphocytes and plasmacytes of the periportal and centrilobular spaces in recipients 8, 13, 18 and 19 (Figs. 15 and 16).
- Loss of hepatic architecture and the presence of mononuclear cells in recipients 12, 13, 15, 16, 18, 19 and 20 (Fig. 17).
- Zones of autolysis with polymorphonuclear cells and bacteria in recipients 13, 16, 18 and 19.
- In recipients 2, 3 and 11 no lesions were observed.

Conclusions.

No problems were encountered regarding the harvesting and perfusion of the donor livers with regard to the techniques employed.

The size and weight of the donor and recipient must be similar. The use of smaller animals as donors led to strictures of the anastomoses due to the difference in the diameters of the vessels.

Features regarding technique in the recipient.

- Because of the difficulty working in the right subphrenic space, two linen sutures were placed in the diaphragmatic muscle lateral to the posterior vena cava. When tied to the parallel arms of an abdominal retractor, these can be used for caudal traction. Thus, the right hemidiaphragm, the subphrenic vena cava and the liver can be immobilized and displaced inferiorly thereby facilitating work on these structures.
- Once the hepatectomy and caval division are complete the suprahepatic anastomosis is difficult to suture due to the short cuff of the vena cava at the diaphragm.[8] In order to overcome this problem, the hepatectomy was performed leaving the vena cava intact requiring slow, meticulous dissection that prolonged the operation considerably.[3] However, if prior to the hepatectomy with caval division the two or three anterior hepatic veins are ligated and divided an adequate caval cuff can be obtained thereby decreasing the operative time.
- With the use of a roller pump in the femoro-jugular bypass, it was possible to control adequately drainage from the inferior vena cava and portal vein into the superior portion of the vena cava. The simple tube in the passive shunt must have an internal diameter of least 4.5 mm in order to function properly.
- The end-to-side anastomosis between the common hepatic artery of the donor liver and the common hepatic artery of the recipient was employed because ligation of the gastroduodenal artery, required for an end-to-end anastomosis, risks serious devascularization of the duodenum and pancreas.[9]
- The task of taking down the side-to-side portacaval anastomosis draining the splanchnic system and cava requires a great deal of time.[6] This is overcome by the temporary occlusion of this anastomosis with a plastic clip that can be applied easily and quickly.
- Determination of bile production and secretion, one of the hepatocellular functions, is accomplished by means of cholecystostomy. In several animals the biliary drainage was diminished due to the reduced caliber of the tubes and technical errors.

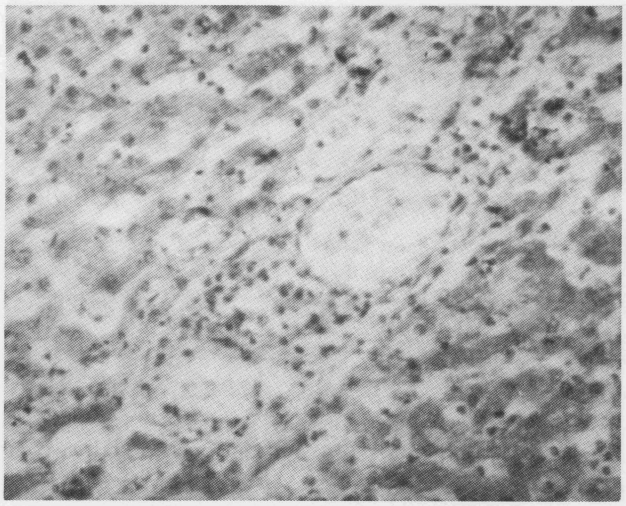

Fig. 15. — Photomicrograph, 260. Portal space with mononuclear cell infiltration.

The exact cause of death in the recipients, usually due to intraoperative and postoperative complications, is difficult to determine (Table I). Intraperitoneal hemorrhage was a manifestation of deficient operative technique such as anastomotic defects and rupture of the hepatic parenchyma.

TABLE I

Probable Causes of Death in 20 Orthotopic Liver Transplant Recipients

Recipient	Probable cause of death
1	intraperitoneal hemorrhage
2	intraperitoneal hemorrhage
3	respiratory insufficiency
4	splanchnic sequestration
5	intraperitoneal hemorrhage
6	right pulmonary atelectasis
7	splanchnic sequestration, arterial thrombosis
8	undetermined
9	gastrointestinal hemorrhage
10	myocardial infarct, pulmonary edema
11	intraperitoneal hemorrhage
12	intraperitoneal hemorrhage
13	hepatic insufficiency
14	gastrointestinal hemorrhage
15	gastrointestinal hemorrhage
16	hepatic insufficiency
17	gastrointestinal hemorrhage
18	hepatic insufficiency, rejection
19	hepatic insufficiency
20	arterial thrombosis

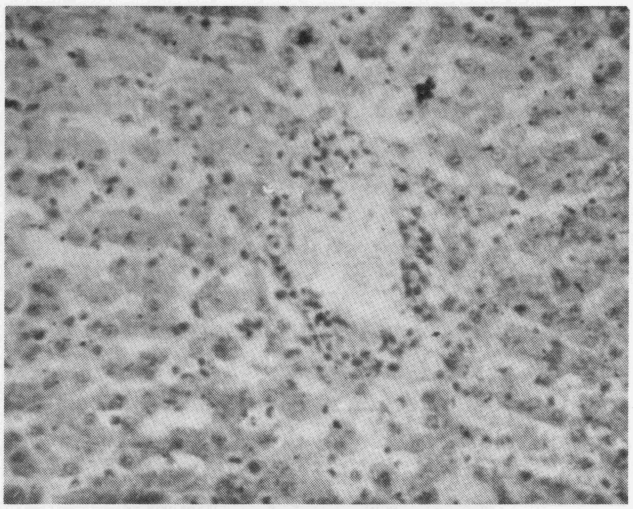

Fig. 16.— Photomicrograph, 260. Centrilobular vein, mononuclear cell infiltration.

Consumptive hypocoagulation with hyperfibrinolysis, as a result of cardiovascular insufficiency and the anhepatic period in addition to physico-chemical injury to the donor liver, could be responsible for capillary hemorrhage in the incision.[4]

Arterial thrombosis caused hepatic dearterialization demonstrated by elevated plasma enzymes. Thrombosis was due to strictures at the anastomoses.

Early deaths were caused by hepatic outflow obstruction manifested clincially by bluish discoloration of the liver and gastrointestinal hemorrhage, at autopsy by firm hepatomegaly and splanchnic congestion and histopathologically by distension and collapse of the sinusoids.[6]

The degree of outflow obstruction,[1,3] in the context of a patent subphrenic anastomosis, directly limits the time of hepatic anoxia. From the first postoperative day, all of the animals had elevated enzymes, a manifestation of hepatocellular damage due to a moderate period of

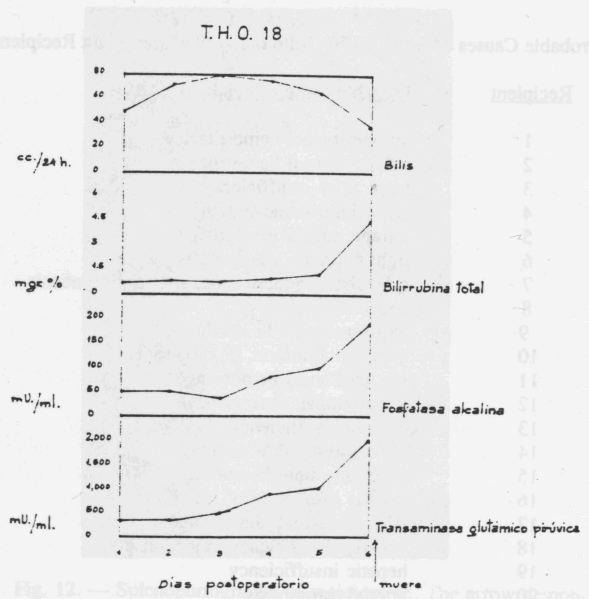

Fig. 18.—Demonstration of the biochemical changes in recipient 18.

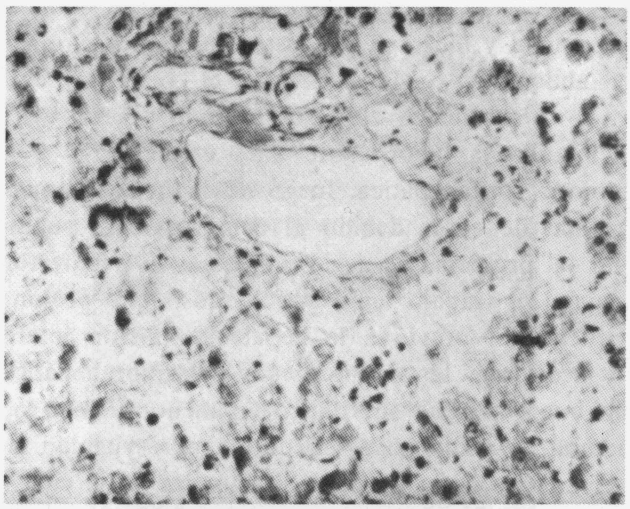

Fig. 17.— Photomicrograph, 260. Loss of architecture and periportal hepatocytes.

ischemia.[5] The animals that died after the fourth postoperative day demonstrated histologic evidence of rejection, i.e., mononuclear cell infiltration and destruction of the hepatic parenchyma.

Vomiting and convulsions were observed in those recipients that died between the third and fourth postoperative days; in these, laboratory results indicated progressive hepatic damage. The pathogenesis of this hepatic insufficiency is obscure.

During the fourth and fifth postoperative days[7] recipient 18 became prostrate and did not eat; transaminase, alkaline phosphatase and bilirubin were elevated and, at the same time, bile formation decreased (Fig. 18).

The syndrome of rejection in recipient 18 is an immunologic phenomenon that injures and ultimately destroys the hepatocyte and results in intrahepatic cholestasis.

With auxiliary liver transplantation[2] survival was better than that achieved in our study. The explanation can be found in the fact that the recipient of an auxiliary liver does not endure an anhepatic period, the period of hepatic anoxia is diminshed because the duration of the procedure decreased so that insufficiency of the auxiliary liver does not cause death of the recipient.

Summary.

The surgical technique employed in performing 20 canine orthotopic liver transplantations is described.

The animals were evaluated by clinical observation, by laboratory and radiologic examination and by autopsy.

The surgical technique is comple and was responsible for several failures.

Hepatic venous outflow obstruction, in conjunction with hepatic anoxia, was one of the main causes of early death in the dogs.

The syndrome of rejection was observed in one dog.

In three dogs, the pathologic anatomy revealed lesions compatible with rejection.

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Scot Denmark

Scot Denmark, the senior author of this experimental study, was a General Surgical Resident on a one-year rotation in the laboratory. He since has become a cardiac surgeon and is in private practice in Dayton, Ohio. Although there were not many animals in this investigation, the work had a profound influence in clinical practice.

Veno-venous bypass without systemic anticoagulation in canine and human liver transplantation

Surgical Forum, 34: 380-82, 1983

Scot W. Denmark, Byers W. Shaw, Jr., Thomas E. Starzl and Bartley P. Griffith

Previous attempts to perform liver transplantation during veno-venous bypass with systemic anticoagulation (heparin) in 12 patients at this medical center resulted in excessive blood loss in six and at least one fatality. The purpose of this study was to test the plausibility and consequences of using a veno-venous circuit without systemic anticoagulation during liver transplantation.

MATERIALS AND METHODS

Orthotopic liver transplantation was performed in ten mongrel dogs (20 to 25 kg) while using the bypass circuit without systemic heparin. Results of hematologic profiles, hemodynamic measurements, and postmortem examination were compared with those in three dogs in which no bypass was used. These three control transplants employed the "cuff" technique described by Cooperman et al.[1] Bypass was initiated with drainage from the divided portal vein and proximally occluded infrahepatic vena cava with return to an external jugular vein. The circuit consisted of a centrifugal pump and flow probe with 3/8-inch Tygon tubing coated with 5% albumin and primed with a physiologic electrolyte solution. Appropriately sized Bahnson caval cannulas were used for the jugular vein and cava, while a fenestrated stainless-steel ventricular vent with 1/4-inch Silastic tubing drained the portal vein. Bypass continued for two to four hours at rates of flow between 400 and 1,500 mL/min. In the clinical trial. Caval drainage was obtained with 7 mm-Gott tubing introduced via the right common femoral vein advanced to the iliac vein; the same size was used for the portal vein, and a 9 mm-piece provided venous return to the patient's left axillary vein. The remainder of the circuit was unchanged.

RESULTS

The use of bypass avoided low cardiac output and portal venous congestion usually associated with caval and portal vein occlusion. Hematologic profiles were not significantly different between groups and indicated a slight decrease in RBCs (5.67 to 5.16 x 10^6/mm^3), WBCs (13.8 to 9.8 10^3/mm^3), platelets (145 to 117.5 x 10^3/mm^3), and fibrinogen (145 to 80 mg%/dL) associated with the bypass. Fibrin split products and monomers were moderately elevated (staph clumping 1 to 128, ethanol gel 1 to 4+). Fibrin threads were seen on the support struts of the pump housing in two studies in which the flow rate decreased below 800 mL/min. Gross and microscopic examination of the lungs failed to show intravascular thrombus. In the clinical trial, PT, PTT, and fibrin monomers increased slightly during bypass, whereas fibrin split products remained unchanged and fibrinogen levels decreased. The patient developed a fatal aspiration pneumonia during the first postoperative week. At postmortem examination, no intravascular thrombus was found at the cannulation sites or in the lung.

DISCUSSION

The use of a bypass circuit in liver transplantation provides several advantages by avoiding severe cardiovascular derangements, particularly hypotension, often associated with caval clamping prior to hepatectomy. At completion of the infrahepatic vena caval and portal anastomoses, blood rich in potassium and hydrogen ions is returned to the systemic circulation from the previously stagnant capillary beds of the lower body and splanchnic circulations. A bypass circuit that decompresses the portal vein and the cava below the liver could prevent the accumulation of potassium and acid which might cause cardiac arrhythmias. Azpeitia et al used several different shunts (without a pump) during liver transplantation in dogs.[2] Partial cardiopulmonary bypass provided hemodynamic stability during human liver transplantations performed by Calne et al.[3] All of these investigators used heparin systemically. In this experiment, veno-venous bypass without systemic anticoagulation preserved the physiologic state during liver transplantation without imposing significant coagulopathy or

thromboembolism and served to stimulate a clinical trial in which bypass cannulas were heparin bonded. The use of this system in one patient was associated with normal cardiac output, portal decompression, and no significant change in coagulation factors, platelet count, fibrin split products and monomers, or thromboelastogram. The study has demonstrated the feasibility of veno-venous bypass without systemic anticoagulation. The experimental and early clinical success has prompted plans for routine clinical use in human liver transplantation.

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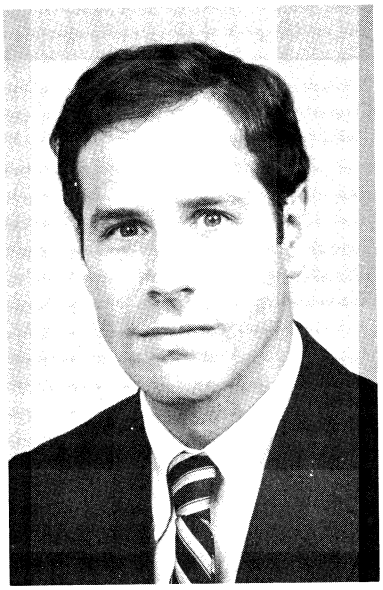

B. P. Griffith

The role of Bartley Griffith, then an Assistant Professor of Cardiac Surgery, in the development of veno-venous bypasses is described in the introduction ot this section. Dr. Griffith has become the head of an internationally known and respected thoracic transplant program at the University of Pittsburgh and is now Associate Professor of Surgery at the age of 39 years.

Veno-venous bypass without systemic anticoagulation for transplantation of the human liver

Surgery, Gynecology & Obstetrics, 160: 270-2, 1985

Bartley P. Griffith, Byers W. Shaw, Jr., Robert L. Hardesty, Shunzaburo Iwatsuki, Henry T. Bahnson and Thomas E. Starzl

A crucial intraoperative period during orthotopic liver transplantation occurs during mobilization of the diseased recipient liver prior to hepatectomy and during the subsequent anhepatic phase of the hepatectomy and implantation of the new liver. During this time, obstruction of the portal vein and inferior vena cava can result in a fall in cardiac output and arterial pressure,[1] consequent hypoperfusion of critical organs, damage to the engorged subdiaphragmatic capillary beds and extensive bleeding from high pressure venous collaterals. These nonphysiologic conditions have been responsible for intraoperative or delayed deaths and have contributed to the lack of wide application of replacement of the liver.[2]

When orthotopic liver transplantation was developed in dogs, bypass techniques were used to shunt blood from the lower to the upper half of the body without pumps and systemic heparinization.[3,4] The clinical application of the passive bypass resulted in the formation of clots in the tubing with consequent passage of multiple "emboli" to the lungs.[5] The use of a pump driven bypass under systemic heparinization was discouraged by major difficulties in reversing the heparin effect in patients with endstage hepatic disease and multiple pre-existing coagulation defects.[2,6,7] We report herein upon a pump driven nonheparin bypass system that was tested in dogs and was subsequently applied in 23 patients who underwent replacement of the liver.

Technique.

The unique components of the circuit include modified heparin bonded Gott aneurysm shunt tubing (argyle Division, Sherwood Medical) for drainage and return cannulae, plus a centrifugal blood pump (Bio-Medicus, Inc.). The cannula to drain blood from the inferior vena cava is advanced through the saphenofemoral venous junction to near the confluence of the common iliac veins (Fig. 1). The cannula which removes splanchnic blood is placed end-on into the transected portal vein (Fig. 1). The fusiform Gott shunts which are 60 centimeters long with an extra side hole at one end, are ideal for drainage. After intravascular insertion, these cannulae are cut where their diameter is large and joined together with a three-eighths inch polished stainless steel Y type connector (Fig. 1). The blood is drained to a centrifugal pump which returns the effluent to the ipsilateral axillary vein (Fig. 1) through a 7 or 9 millimeter Gott cannula without a side hole. Connections of the cannulae to the blood pump and a flow meter are made with short segments of uncoated three-eighths inch polyvinyl chloride tubing.

The entire curcuit may be primed an debubbled on the operative field. After connecting the sterilized components, the drainage cannulae are submerged in a basin of saline solution. A regulated wall suction is then applied to the tip of the return cannula so that priming is completed as the saline solution is drawn through the circuit components. The disposable blood pump head may then be passed off the the field and connected to its motor console. Alternatively, the cannulae may be individually primed by a bulb type syringe and connected to the remainder of the circuit which has been primed by the extracorporeal perfusion team in a closed loop manner. While the former method does not require input from a perfusionist, the latter has caused the least operative interruption and is currently preferred.

The rotation of the centrifugal blood pump is adjusted to deliver the maximum blood return at the minimal revolutions per minute. In adults, total flow has averaged 4 liters per minute and has ranged between 1.5 and 6.0 liters. In many patients, the portal circulation has contributed more than one-half of the total flow. The veno-venous bypass has often been initiated prior to extensive retrohepatic resection, and its average duration of 100 minutes (a range of 70 to 158) has, therefore, included the time required for the completion of recipient hepatectomy plus implantation of the donor graft.

Venous reservoirs are not needed in this system. All circuit tubing is shortened as much as possible to minimize exposure to foreign surfaces. Except for the rare formation of fine fibrin strands on the rims of the connectors and on the pump cone supports, there has been no evidence of *ex vivo* thrombosis. In one patient, a soft iliac vein thrombosis was removed with the drainage cannula, but there has been no clinical or autopsy evidence in any patient of venous embolism. Component blood replace-

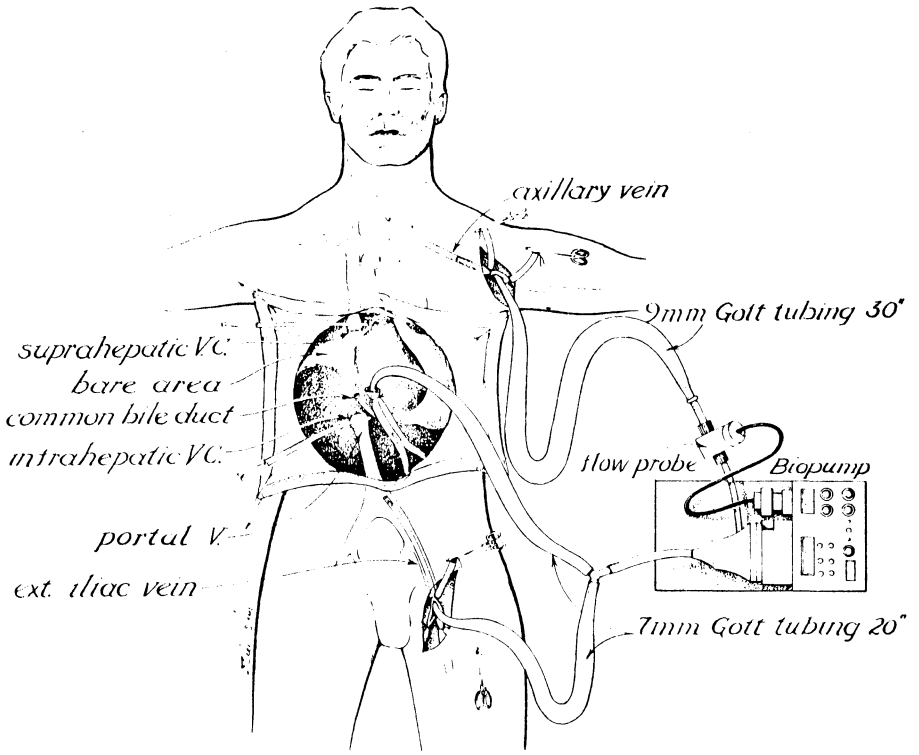

Fig. 1.— Anhepatic stage of transplantation of the liver with cavoportal to axillary bypass circuit.

ment during the period of bypass has actually improved the pre-existing coagulopathy during the bypass in spite of a tendency for the platelet counts to fall during this time.

So far, this system has been used only in adults. Children have been found to tolerate the combined portal and venacaval occlusion far better than adults, and it is possible that the system should be used in younger liver recipients only when indicated by vasomotor instability during test cross clamping. In adults, the advantages of bypass are so great that it has become a routine part of transplantation of the liver.

Summary.

A technique of veno-venous bypass without heparin has been developed for use during the anhepatic phase of transplantation of the liver. With this method, the ability to compress the temporarily obstructed vena caval and portal venous systems has made hepatic transplantation an easier procedure.

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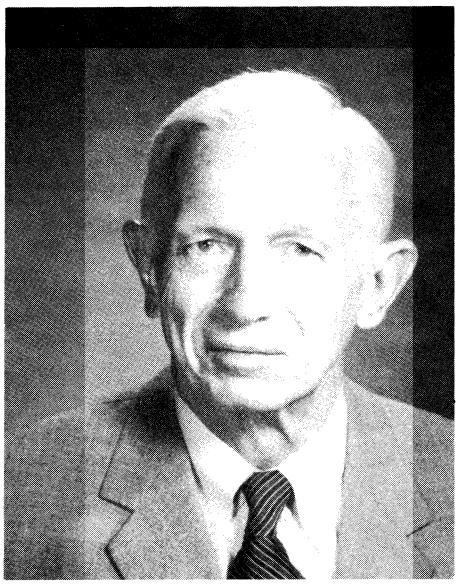
Byers W. Shaw, Jr.



Henry T. Bahnson — "Hank" Bahnson was Chairman of the Department of Surgery at the University of Pittsburgh for 25 years and retired in September 1987. He was one of the pioneer heart surgeons at the John Hopkins Hospital nearly 40 years ago. His contributions to the veno-venous bypass technology are described in the introduction to this section.

Byers Shaw was the Fellow on the Pittsburgh Transplantation Service at the time the veno-venous bypass technique was worked out and applied clinically. His important role in this technical advance was described in the introduction to this section. Dr. Shaw has become one of the most experienced and best liver transplant surgeons in the world. He is now Professor of Surgery and Chief of Transplantation at the University of Nebraska, Omaha.

Venous bypass in clinical liver transplantation

Annals of Surgery, 200: 524-34, 1984

Byers W. Shaw, Jr., Douglas J. Martin, Jose M. Marquez, Y. G. Kang, Alan C. Bugbee, Jr., Shunzaburo Iwatsuki, Bartley P. Griffith, Robert L. Hardesty, Henry T. Bahnson, Thomas E. Starzl

A venous bypass technique (BP) that does not require the use of systemic anticoagulation is used routinely at our institution in all adult patients during the anhepatic phase of liver transplantation (LT). Complete cardiopulmonary profiles were obtained in a subset of 28 consecutive cases. During the anhepatic phase while on bypass, mean arterial pressure, central venous pressure, and pulmonary arterial wedge pressure were maintained at prehepatectomy levels. Oxygen consumption fell secondary to a decrease in temperature and the removal of the liver. Consequently, cardiac index fell without an increase in arterial-venous O2 content difference, reflecting adequate tissue oxygenation. Compared with 63 patients in a previous series given LT without bypass (NBP), the 57 total BP patients experienced better postoperative renal function ($p<0.001$), required less blood use during surgery ($p < 0.01$), and had better survival 30 days after LT. The equivalency of 90-day survival in these groupa results from the lack of effect of BP on the long term survival of patients considered at high risk for metabolic reasons. BP patients at high risk for technical considerations, however, survived LT whereas NBP patients did not. BP offers other advantages important in establishing LT as a service-oriented procedure.

The dramatic impact of cyclosporine on survival following liver transplantation has been widely reported.[1-4] Yet despite extensive experience with the operation during the preceding 17 years, only a few important technical improvements were reported to have significantly enhanced survival.[3,5-8] In fact, during the first 3 years in which cyclosporine was used, mortality related to a difficult intraoperative course remained a disturbing problem. For the most part, these difficulties centered around the anhepatic phase and repeatedly underscored the need for an effective method of venous bypass. The need for the development of new methodology was clearly demonstrated by the severe penalty imposed by the requirement for systemic heparinization during a trial of venous bypass using conventional techniques in 12 patients during the summer of 1982. The development in the laboratory of a heparin-less bypass system was reported by Denmark et al.[9] and the initial clinical trial using these techniques presented by Griffith et al.[10] The effectiveness of this method of venous bypass is the subject of the current study.

Methods

Between February 15, 1983 and March 22, 1984, a total of 57 adults were treated with orthotopic hepatic transplantation at the University of Pittsburgh using venous bypass without the use of systemic anticoagulation. The technique used is essentially that described by Griffith with only minor changes. In particular, the use of a 9-mm Gott (Argyle Division, Sherwood Medical, St. Louis, MO) shunt for cannulation of the portal vein and a 7-mm shunt for the axillary vein has proven to be a more satisfactory alternative to the reverse situation described in the previous paper.[10] The femoral vein is cannulated with a 7-mm Gott shunt via a cut-down on the saphenous vein.

The femoral and portal vein cannulae come together at a metal Y-connector, which is in turn attached to 3/8-inch polyvinyl chloride tubing (Tygon, Norton Industrial Plastics, Akron, OH), which leads to a conical, centripetal force pump head (Bio-Medicus, Inc., Minnetonka, MN). The return flow is to the axillary vein. The system is a completely closed one that does not require a reservoir in the circuit. An electromagnetic flow meter (Bio-Medicus, Inc., Minnetonka, MN) is attached to the outflow of the pump head. Flows are maintained at 1000 ml/min or more, a rate that was defined in the laboratory as that below which formation of clots and platelet aggregates were more likely to occur. Once bypass flow has been established at a satisfactory rate, the vena cava below and above the native liver is clamped and the liver excised. Hemostasis in the liver bed can then be obtained, if necessary. The portal venous and lower caval systems are decompressed so that, in general, venous hypertension does not exacerbate the bleeding at this point.

The revascularization of the new liver then occurs in a routine manner.[3] The usual sequence involves sewing the upper vena cava anastomosis followed by the lower caval anastomosis. The liver is then flushed out with 300 to 500 ml of cold saline via the portal vein cannula. This step flushes the preservation fluid, high in potassium, out of the liver. It also fills and evacuates air from the vena cava. The patient is placed in a Trendelenberg position and the fluid allowed to escape through the anterior part of

the lower caval anastomosis. Whether the portal vein or arterial anastomosis is completed next is dependent upon a number of variables. In general, 60 to 80 minutes are allowed for ther anastomoses before some flow is returned to the liver. In those situations where clamping the portal vein side of the bypass circuit (in order to perform the portal vein anastomosis) results in dropoff of the flow below 1000 ml.min, the bypass should be discontinued to minimize the chance of thrombus formation. If the arterial anastomosis has been completed by this time, the arterial and caval clamps can be released prior to decannulating the portal vein and coming off bypass. The proper choice for sequencing these steps must be determined by the surgeon for each situation.

Intraoperative Monitoring.

Cardiopulmonary data were obtained using a multiple lumen pulmonary artery catheter. In the subset of patients for whom complete profiles were obtained, a four-lumen catheter employing an oximeter at the distal tip (Oximetrix, Inc., Mountain View, CA) was employed. Complete cardiopulmonary profiles were obtained prior to the induction of anesthesia, after anesthesia had been established but before significant fluid losses had occurred, just before initiating the bypass, during the bypass, and shortly after revascularization of the liver. Oxygen consumption was calculated using the Fick equation. Coagulation data were obtained routinely on all patients using a thromboelastogram.

Renal Function.

The mean serum creatinine of all patients was determined immediately prior to undergoing liver transplantation and then after surgery. Comparisons were made between the preoperative values and the highest postoperative value with 72 hours of transplantation. The means were compared using the paired and unpaired Student's t-tests. The number of patients in the bypass and non-bypass groups who were not on dialysis prior to surgery but who required dialysis within the first 7 days of transplantation were determined and compared using the chi square test. Patients with preoperative renal failure or preoperative requirements for dialysis were eliminated from this latter comparison. A subset of 30 consecutive patients were monitored for urine output during surgery.

Blood Use.

Figures regarding the number of units of blood components used during transplantation operations were kept by the Central Blood Bank of Pittsburgh. Mean blood use was compared using the Student's unpaired t-test and the median values by the rank-sum test.

Mortality and Survival.

Life-table analysis was used to determine survival of the various groups and these values compared by the method of Greenwood[11] using the standard errors of the survival possibilities for each interval. Minimum follow-up on all patients was 35 days. A total of 63 and 57 patients in the non-bypass and bypass groups, respectively, were used for survival calculations.

Heparinized Bypass from June 15-September 6, 1982.

Twelve patients, nine of whom were adults, underwent liver transplantation using a conventional bypass system that necessitated systemic antocoagulation with heparin. These are the subject of a brief comparison with the current system.

TABLE 1. *Indications for Liver Transplantation*

	No Bypass	Bypass
Postnecrotic cirrhosis	25	17
Primary biliary cirrhosis	12	16
Sclerosing cholangitis	6	10
Malignancy	6	5
Budd-Chiari syndrome	4	0
Secondary biliary cirrhosis	4	1
Alpha-1-antitrypsin deficiency	4	1
Wilson's disease	0	4
Miscellaneous	2	3
Total	63	57

TABLE 2. *Cardiopulmonary Profiles*

	Pre-bypass		Bypass		
Variable	Mean	SD	Mean	SD	P
T(°C)	34.9	.98	33.8	1.16	0.0002
HR (bpm)	85.6	14.3	84.1	14.2	
MAP (mmHg)	75	17.1	83	17.6	
PAO (torr)	12.0	4.2	10.5	3.3	
CVP (mmHg)	9.9	4.4	9.2	4.9	
pCO_2 (torr)	33.3	5.2	29.9	4.8	0.0125
pO_2 (torr)	380	92.4	398	91.6	
SaO_2	0.9854	0.0915	0.9896	0.0160	
PvO_2 (torr)	65.5	16.3	70.8	21.5	
SvO_2 (torr)	0.904	0.0598	0.927	0.0496	
Hgb (g/dl)	9.62	1.16	10.2	1.42	
pHa	7.42	0.07	7.40	0.08	
CI ($L/min \cdot m^2$)	4.37	1.44	3.44	1.09	0.0079
SI	51.7	15.9	41.4	13.6	0.0121
RVSWI	5.11	3.21	3.52	3.17	
LVSWI	43.4	14.3	39.7	11.4	
SVRI	443.7	233	660	366	0.0109
PVRI	34.3	23.9	44.1	39.1	
a-vDO_2	2.04	.81	1.87	.59	
VO_2/m^2	83.0	24.7	61.7	20.5	0.0009
O_2 transport	1087	391	917	342	

HR–heart rate; MAP–mean arterial pressure; PAO–pulmonary arterial wedge pressure; CVP–central venous pressure; pCO_2–partial pressure carbon dioxide; pO_2–partial pressure oxygen; SaO_2–arterial oxygen saturation; PvO_2–mixed venous oxygen pressure; SvO_2–oxygen saturation pulmonary artery; Hgb–hemoglobin; pHa–arterial pH; CI–cardiac index; SI–saturation index; RVSWI–right ventricular stroke work index; LVSWI–left ventricular stroke work index; SVRI–systemic vascular resistance; PVRI–pulmonary vascular resistance; a-vDO_2–arterial-venous oxygen content difference; VO_2/m^2–oxygen consumption.

Results

From March 9, 1980 to March 22, 1984, a total of 129 adult patients, 12 at the University of Colorado and 117 at the University of Pittsburgh, underwent orthotopic hepatic transplantation. Sixty-three of these patients were treated without the use of any venous bypass technique, nine with bypass technique utilizing systemic anticoagulation, and 57 utilizing venous bypass without systemic anticoagulation. The indications for transplantation are shown in Table 1.

Cardiopulmonary Data.

Flow in the bypass system ranged from 1 to 13 liters per minute, with a mean of 2.77 (±0.827) L/min. Average bypass time was 91.2 (±19.7) minutes. Complete cardiopulmonary data were obtained in 28 consecutive cases between March 30 and August 16, 1983. These data are presented in Table 2. The pre-bypass values are the means (±SD) of those obtained after induction of anesthesia but before devascularization of the native liver. The bypass data are the means (±SD) of the data obtained during the actual anhepatic state while patients were undergoing venous bypass. These data show that the oxygen consumption (VO_2/M_2) fell secondary to a decrease in temperature (T) and removal of the liver. Consequently, cardiac index (CI) fell without an increase in arterial-venous oxygen content difference (a-vDO_2), reflecting adequate tissue oxygenation. In addition, systemic vascular resistance (SVRI) fell significantly while mean arterial pressure (MAP), central venous pressure (CVP), and pulmonary arterial wedge pressures (PAO) did not change.

Renal Function.

Table 3 reveals that renal function changed to a significant degree in

TABLE 3. *Comparisons of Postoperative Renal Function*

	No Bypass (N = 38)	Bypass (N = 48)	p
Preoperative mean serum creatinine	1.65 ± 1.85	1.23 ± 1.43	
	p < 0.005	p < 0.05	
Postoperative mean serum creatinine*	3.00 ± 2.70	1.51 ± 1.27	
Mean change	+1.29 ± 1.95	+0.35 ± 0.90	p < 0.001
Patients requiring dialysis during first postoperative week	6	0	p < 0.01

* Maximum value in first 3 postoperative days.

both the patients with bypass and those without. Of note is that the increase in creatinine in the non-bypass group (+1.29 ± 1.95 mg/dl) is significantly greater (p<0.001) than that seen in the bypass group (+0.35 ± 0.90 mg/dl). Complete anuria or marked oliguria was not an infrequent occurrence during the anhepatic phase in the previous e perience with liver transplantation.[3] Urine output during the anhepatic phase is maintained quite well in patients on bypass, with a mean urine flow of 187 (± 249) ml/hour (range 0-1360 ml/hour). Complete anuria or marked oliguria was not an infrequent occurrence during the anhepatic phase in the previous experience with liver transplantation.[3] Urine output during the anhepatic phase is maintained quite well in patients on bypass, with a mean urine flow of 187 (± 249) ml/hour (range 0-136 ml/hour). Complete anuria was experienced in only one patient in the bypass group. In addition, marked hematuria, a postoperative occurrence in 10% to 15% of non-bypass adults, has been absent in the bypass group.

Blood Use.

Data were compared for the total blood use during the transplant operation of 36 patients using venous bypass and a total of 43 without bypass. These data are presented in Table 4. The range in blood use of the non-bypass group was 3 to 251 units of packed red blood cells (PRBC) and was 5 to 157 units PRBC for the bypass group. The median blood use was 27 for the non-bypass group *versus* 16 for the bypass group (p<0.05). Mean blood use was calculated by eliminating 10 cases from each group. The five lowest and five highest numbers were withdrawn from the comparison because of the wide standard deviations, which did not allow for a representative comparison. The mean loss in the non-bypass group (32.7 ± 25.1 units PRBC) was higher (p<0.01) than in the bypass group (18.9 ± 8.0 units PRBC). An examination of Table 5 reveals that the mean blood loss with the heparinized bypass sytem was 53.1 ± 33 units PRBC with a range of 10 to 94 units, significantly higher than the non-heparin bypass group (p<0.001) or the non-bypass group (p<0.05).

Hospital Stay.

The number of days that patients remained in the hospital from the date of transplantation to the date of discharge are compared for the two groups in Table 6. The mean length of stay for the non-bypass group was 56.4 (± 35.1) days *versus* 51.1 (± 28.9) days for those bypassed (p>0.50). If all patients whose prolonged hospital stay was secondary to retransplantation were eliminated, the length of stay is still not different between these groups (p>0.05).

TABLE 4. *Packed Red Blood Cell Requirement (Units)*

	No Bypass (N = 43)	Bypass (N = 36)	p
Range	3-251	5-157	
Median	27	16	p < 0.05
Mean	32.7 ± 25.1	18.9 ± 8.0	p < 0.01

TABLE 5. *Experience with Venous Bypass using Systemic Anticoagulation, June 25, 1982 to September 6, 1982 (Nine Adult Patients Undergoing 11 Operations)*

Operative deaths	3/9 (33%)
Mean blood loss (units PRBC)†	53.1 ± 33.0* ± SD
Range	10-94
30-day survival	5/9 (55.6%)
90-day survival	2/9 (22.2%)‡

* p < 0.05 compared to no bypass; p < 0.01 compared to non-heparinized bypass.

† PRBC-packed red blood cells.

‡ Each living, 22 months after surgery.

Bypass and Retransplantation.

The requirements for retransplantation are similar between the bypass and non-bypass groups, with 14 patients in the former and 12 in the latter group requiring retransplantation. The survival following retransplantation is not significantly different at either 1 month or 3 months after surgery.

Mortality and Survival.

The actuarial survival curves for the non-bypass and bypass gorups are shown in Figure 1. All patients in each group were followed for a minimum of 30 days after surgery and included 57 bypass and 63 non-bypass patients. Survival at 30 days was 91.1% for the bypass group compared to 73% for the non-bypass group (p<0.004). At 90 days, however, survival in the bypass group (73.2%) was not significantly different from that in the group without bypass (68.3%) (p>0.05). Six of the patients in whom bypass was not used died on the operating table. The one operative death occurring in the bypass group was the result of an irreparable injury to the donor liver. That patient experienced no cardiodynamic instability during the hepatic revascularization. Table 5 reveals that three of nine patients bypassed using anticoagulation died in the operating room, and a fourth expired before 30 days. Only two of the nine patients survived beyond 90 days and both were living 22 months after surgery at the conclusion of the study.

An examination of the causes of death in both groups reveals that they are quite similar. Sepsis, accompanied by multiple organ failure and, in particular, liver failure, was universal. In an attempt to understand why the significant difference in survival at 30 days was no longer present by 90 days, patients were classified into three groups. Status 1 patients are stable, relatively low-risk candidates, including those who are primarily outpatient care-dependent with only infrequent hospitalization. Nutritional status is good to excellent. A history of significant esophageal variceal bleeding but with rapid recovery would not exclude a patient from this group. Status 2 patients are those who require frequent hospitalization, who have advanced cirrhosis with moderate to severe ascites, occasional episodes of grade 3 to 4 encephalopathy, recurrent episodes of variceal bleeding with attendant worsening of encephalopathy, and those whose life-style has been significantly altered by their disease. Status 3 patients were the very high-risk group. The criteria for which patients would be placed into this group are either metabolic or technical. All of the metabolic group patients require hospitalization, most in the intensive care unit. All have frequent bouts of grade 3 to 4 encephalopathy. Malnutrition is severely advanced; ascites and hypoalbuminemia are extreme. Some patients with acute hepatic decompensation secondary to toxic agents were

TABLE 6. *Postoperative Hospital Stay (Patients Discharged in Pittsburgh)*

	No Bypass (N = 30)	Bypass (N = 37)	
Range	16-145 days	21-165 days	
Median	51 days	42 days	p > 0.05
Mean	56.4 ± 35.1	51.1 ± 28.9	p > 0.50

placed under this category by virtue of their emergent situation. In the current study, nine of the 26 Status 3 patients (four in the non-bypass, five in the bypass group) were in stage 4 coma in an intensive care unit setting with severe renal dysfunction and requiring ventilatory support. Technical considerations that would require a Status 3 classification include a previous portacaval shunt, thrombosed portal vein, or multiple previous abdominal surgeries involving the liver or bile ducts. Four patients in the non-bypass and two in the bypass group were so classified.

A breakdown of the survival curves based on this assignment of status is depicted in Figures 2, 3, and 4. In Status 1 patients, eight of nine non-bypass and all six bypass patients remain alive at the end of the study. Among 41 Status 2 patients without bypass, 30-day and 90-day survival is 88% and 76%, respectively, compared to 97.2% and 85.2% for the same intervals among 38 Status 2 patients treated with bypass. Thirteen patients were classified as Status 3 in both non-bypass and bypass groups. Survival at 30 days was 31% for the non-bypass group compared to 61.5% of the bypass group ($p<0.01$). However, mortality in the bypass group between 30 and 90 days was significantly higher ($p<0.05$) than in the non-bypass group, so that survival by 90 days was 31% and nine per cent in the non-bypass and bypass groups, respectively (<0.05). Among the four patients classified as Status 3 in the non-bypass group because of technical considerations, three were operative deaths and the fourth died in less than 48 hours after surgery. Both of the technical Status 3 by-pass patients are alive 2 and 6 months after surgery.

Discussion

Despite the landmark improvements in survival rates among liver transplant recipients provided by the introduction of cyclosporine,[1-4] early mortality and morbidity related to a difficult intraoperative course remained a significant problem. Approximately 50% of all patients who died within 1 year following transplantation were lost by the end of the first 30 days. In addition, operative mortality among the first 63 adult patients treated with cyclosporine was 9.5%.

Historical Backgound.

Physiologically and sometimes technically, the most difficult period of the actual transplant operation is the anhepatic phase, when the native liver has been removed. Cross-clamping the portal vein and the abdominal portion of the inferior vena cava causes severe problems. The first results from the loss of return to the central venous system of the large volume of caval flow from the abdomen. The second stems from the hypertension that develops in the obstructed portal and systemic venous beds. The third occurs at the time of liver revascularization when stagnant blood from the obstructed venous beds, sometimes rich in acid and potassium, is suddenly returned into the systemic circulation.

Previous studies revealed that the typical response to the anhepatic phase is a 50% reduction in cardiac output accompanied by a marked increase in systemic vascular resistance.[12] More recent experience has shown that the response can be quite variable. In young, otherwise healthy individuals it is similar to what occurs in severe acute hypovolemia. Although arterial blood pressure is fairly well maintained, cardiac output is reduced to as much as one-fifth of the preocclusion levels while systemic vascular resistance increases three to four fold. Patients with preexisting cardiac dysfunction, or older patients, do not tolerate the loss in central venous volume and marked increase in vascular resistance well and can develop cardiac failure, marked hypotension, cardiac arrhythmias, and even cardiac arrest. Even patients with more resilient cardiac function will sometimes require the infusion of large volumes of fluid (or blood products) into the central venous system in order to compensate for the marked fall in preload. The penalty for this is severe fluid overload at the time of liver revascularization when normal venous return is restored.[3]

The occlusion of portal venous outflow is tolerated to quite variable degrees as well. Clamping the portal vein in the normal dog results in the death of the animal within 20 to 30 minutes.[12,13] Cirrhotic dogs, however, can tolerate portal vein clamping for over 1 hour.[14] Clamping the portal vein in normal man causes fewer problems, either because of a more extensive collateral network or because of the normal lack of bacterial flora in human portal blood.[15,16] Even so, the presence of portal hypertension and extensive collateral circulation in humans with cirrhosis probably provides an advantage similar to that in the canine cirrhosis model.[3] This has been particularly evident when long periods (greater than 1 hour) of portal occlusion have been necessary in patients without portal hypertension, such as those with primary malignancies. The degree of gut swelling and petechial hemorrhage in the bowel wall has been much more severe than that seen in cirrhotics. Frank gastrointestinal hemorrhage has not been an infrequent occurrence in this setting.

In the normal dog model, various methods are used to keep portal occlusion times as brief as possible. These include the classic method of end-to-side portacaval shunt combined with a femoral-to-jugular venous bypass cannula[12,13] or the use of the "cuff" technique for vascular anastomoses originally used by Kamada[17] in rats and employed for the normal dog model by Monden.[18]

The original human trials at Denver involved the use of venous bypasses in anticipation of problems similar to those seen in dogs.[19] But the inordinate incidence of embolic phenomena accompanying the use of passive shunts led to their abandonment when certainty evolved that humans tolerated the anhepatic phase without venous decompression much better than dogs.[15,16] Calne has selectively used partial cardiopulmonary bypass in an attempt to support patients during the anhepatic phase, with varying degrees of success.[20]

A trial of venous bypass using a conventional cardiac bypass apparatus was undertaken in Pittsburgh in the summer of 1982. Although physiologic advantages during the anhepatic phase were quite evident, these were outweighed by the severe penalty imposed by the need for anticoagulation. This is graphically demonstrated in Table 5. Attempts at minimizing the use of heparin provided no improvements and the technique was soon abandoned.

But the need for some sort of bypass technique remained all too evident by the recurring nightmares of the anhepatic phase in a number of patients. Hence, a trial of a method that would not require the use of systemic anticoagulation was undertaken in the laboratory using the normal dog model. The success of these experiments, as mentioned earlier, was reported by Denmark[9] and the initial clinical experience presented by Griffith.[10]

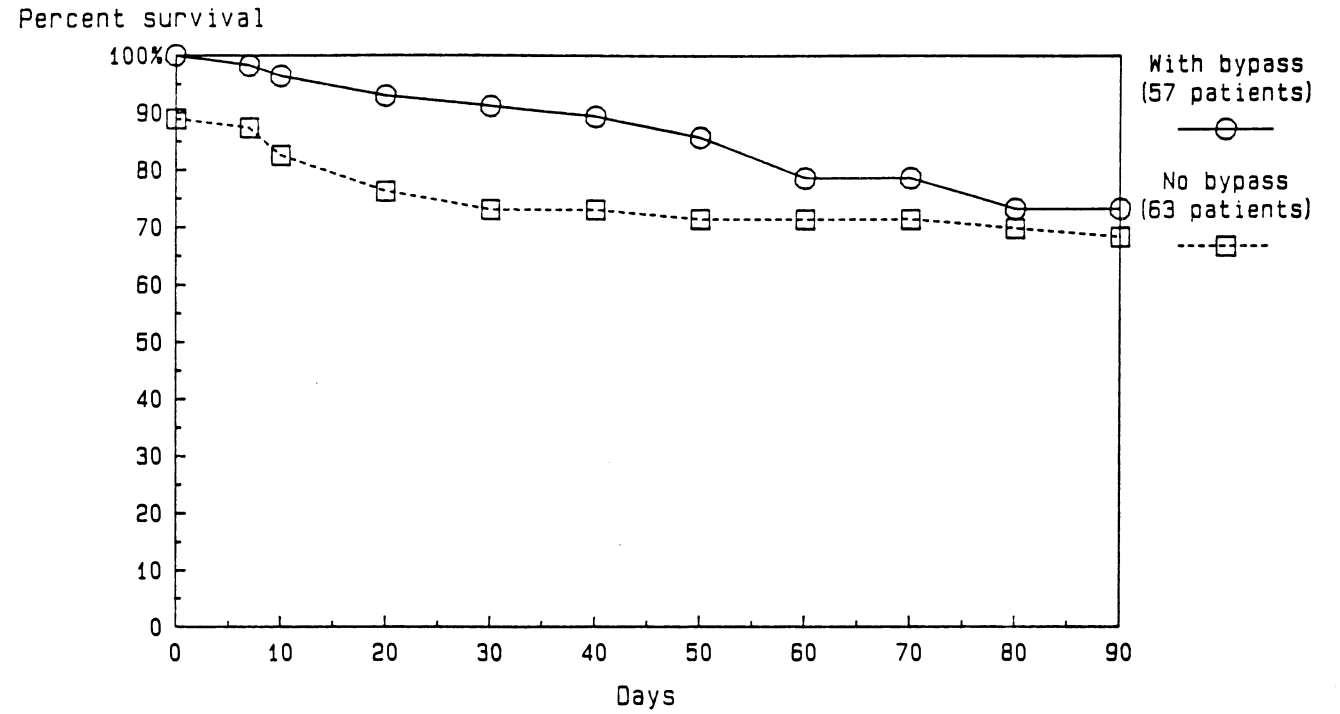
Hemodynamic Advantages.

The data presented in the current study demonstrate the degree to which normal physiology is restored by the use of venous bypass system. Cardiac filling pressures (as measured by a pulmonary artery catheter) are supported, and although the cardiac index falls, it does so to a much lesser degree than it does without bypass and apparently as an appropriate response to decreased demand. This latter contention is supported by the observation that at the same time that cardiac output has fallen, oxygen consumption also decreases while the mixed venous oxygen content does not change significantly. One possible explanation for this is the removal of the liver, a large metabolic machine, from the system. But in addition, the patient's core temperature falls significantly, further lowering metabolic demand. This fall in temperature has been reported before.[21] It was attributed to a variety of causes in the previous report, including long operating times, the presence of the cold (4-10° C) donor liver in the abdomen while the vascular anastomoses were completed, and the revascularization of the donor liver when cold preservation solution is flushed out of the liver and into the systemic circulation by the restored blood flow. The technique of venous bypass used currently is a closed system without a heat exchanger in the circuit. Additional cooling of blood undoubtedly occurs as it passes through the bypass circuit. Patient core temperature falls continuously during the bypass phase and reaches a nadir just after liver revascularization.

The high volume of blood returned to the heart from the venous beds that otherwise would be obstructed is further evidenced by the high flow rates obtained in the bypass circuits. The mean flow for all patients of 2.77 ($\pm$0.827) L/min represents 55% of the mean cardiac output obtained for these patients during the bypass phase.

Renal Function.

The smaller increase in serum creatinine level and the virtual elimination of the requirement for hemodialysis during the early recovery phase in the by-pass group is encouraging. Previous difficulties, evident in the current study by the mean postoperative serum creatinine in the non-bypass group of 3.0 ($\pm$2.7) mg/dl and by the requirement among six patients (without overt renal dysfunction before surgery) for hemodialysis



p < 0.01 at 7 days
p < 0.01 at 30 days

Fig. 1.— Ninety-day actuarial survival for liver transplants performed with and without venous bypass.

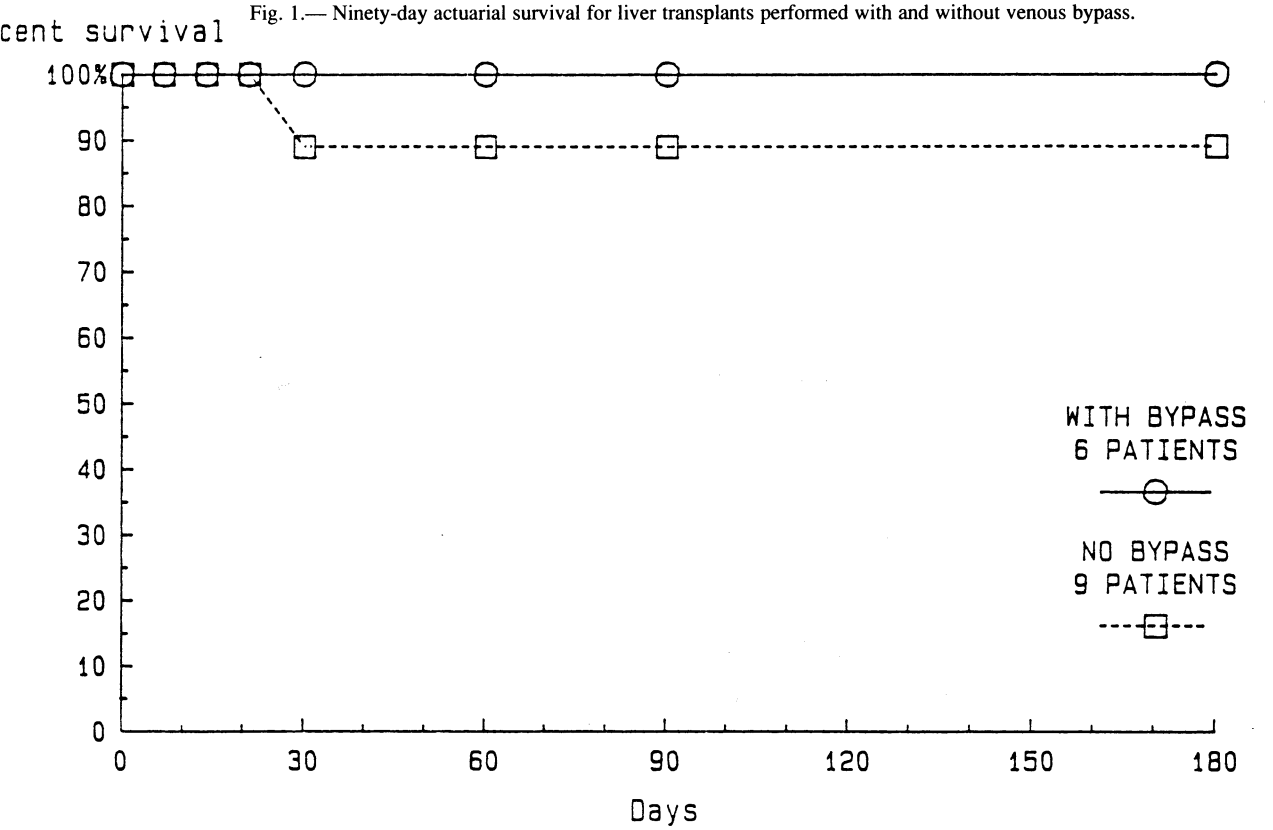


Fig. 2.— Actuarial survival of Status 1 patients with and without venous bypass.

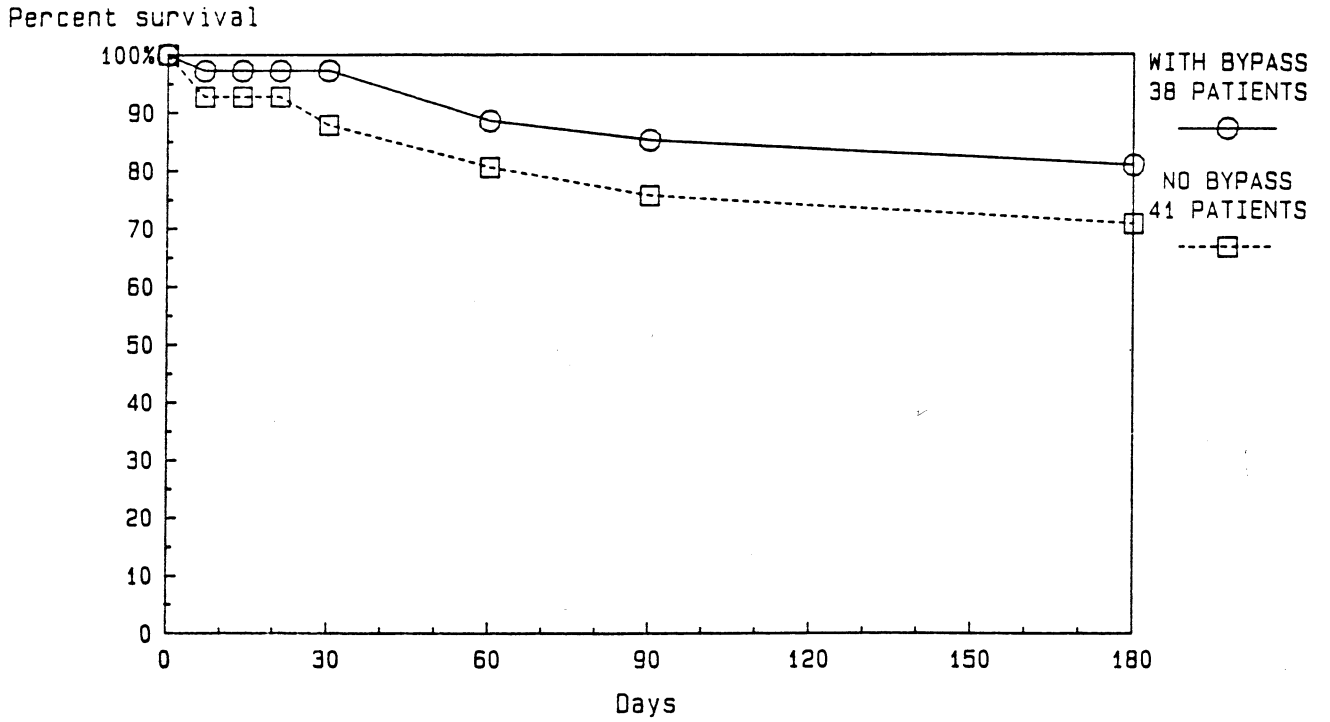
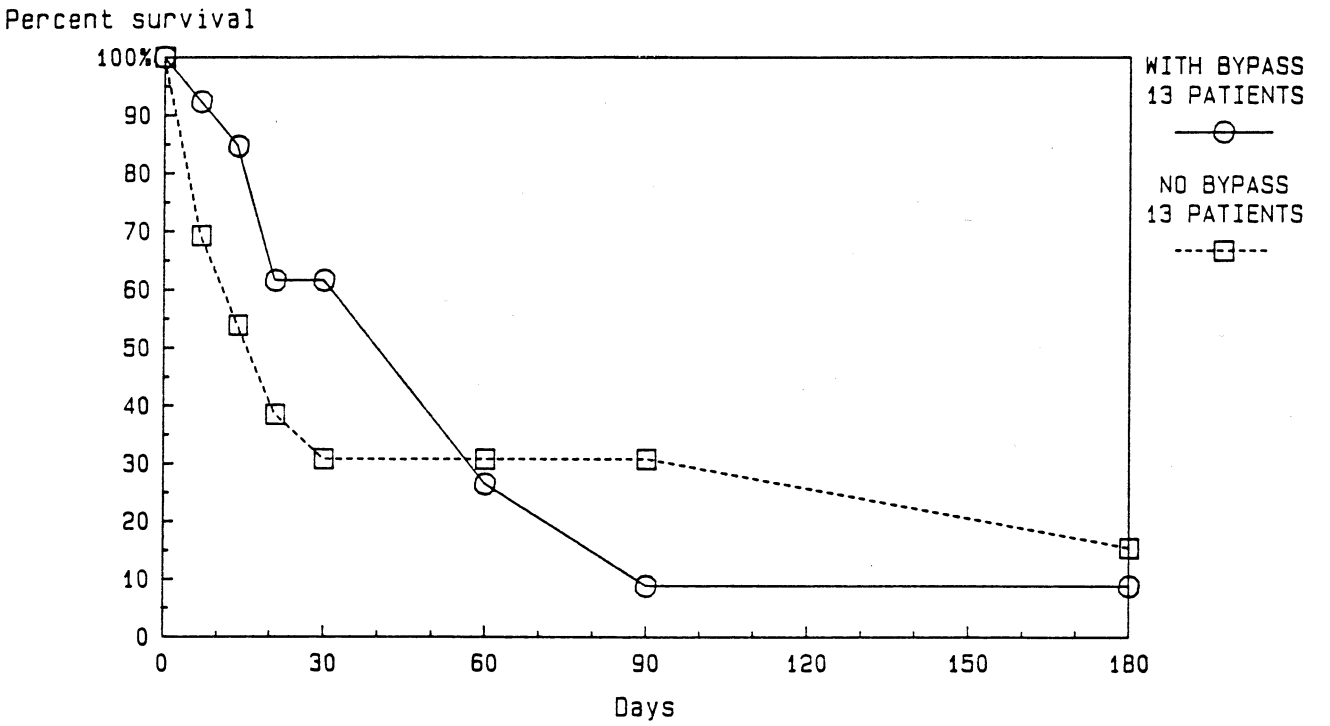


Fig. 3.— Actuarial survival of Status 2 patients with and without venous bypass.



p < 0.01 at 30 days
p < 0.05 at 90 days

Fig. 4.— Actuarial survival of Status 3 patients with and without venous bypass.

during the first week following transplantation, are undoubtedly related to renal damage occurring primarily during the anhepatic phase when the suprarenal vena cava is completely occluded.

Blood Use.

The lower blood use documented since routine venous bypass was instituted is explained by several factors. The most important results from the complete lack of venous hypertension in the systemic and splanchnic beds during the anhepatic phase. Without bypass, this hypertension continues to increase and hemorrhage can accelerate markedly during the anhepatic phase. The bypass also allows for a certain period of time (15-60 minutes) of meticulous effort at hemostasis in the hepatic bed after the liver is out of the way.

Length of Postoperative Recovery.

The use of bypass had no effect on the length of hospital stay. This is true even if one eliminates from consideration all patients whose recovery was complicated by the necessity for a second or third transplant. A bias may be present in these figures since the routine monitoring of daily cyclosporine blood levels was instituted after the introduction of venous bypass for all adult recipients. These blood levels demonstrated surprisingly variable cyclosporine absorption, which necessitated prolonged hospital stays for a number of patients who might otherwise have been discharged earlier in the pre-bypass era.

Mortality and Survival.

An examination of Figure 1 reveals that although intraoperative mortality and the postoperative mortality up through the first 30 days is markedly less in the bypass group, by 90 days this difference is no longer significant. The reason for this apparent parado is revealed by Figures 2, 3, and 4. Four of 13 patients in the high risk group (Status 3) who were not bypassed died in the operating room in contrast to no operative deaths in the bypass Status 3 group. At 30 days, the improved survival continued but was overshadowed by a markedly increased mortality in the bypass group during the next 60 days when compared to the non-bypass group. Seven of these 13 bypass patients required retransplantation, two in whom rejection followed periods of satisfactory liver function, the other five without every [sic] obtaining satisfactory hepatic graft function. Two of these patients died before 30 days, the other five between 30 and 90 days. In the non-bypass group, two patients required retransplantation, both of whom subsequently died. More importantly, only two of the bypass patients were classified Status 3 because of extreme technical considerations and these are the only two survivors from this category. In contrat, four of the 13 non-bypass Status 3 patients were so categorized for technical concerns. Three died during surgery and the fourth 2 days later.

The causes of death among those in both groups who survived beyond the first 2 weeks after surgery are virtually indistinguishable. All had severe hepatic dysfunction and developed sepsis with subsequent multiple organ failure. Overall, this is an extremely high-risk group in whom the application of venous bypass results in improvements in early survival but appears to have little impact on long-term results. An extremely important exception to this conclusion must be made in the case of those patients determined to be high risk for technical reasons. Both surviving patients in the bypass group would almost certainly have been operative mortalities without the use of bypass. In fact, in the pre-bypass era, one of these patients would have been excluded from candidacy for liver transplantation by virtue of having a known portal vein thrombosis.

Complications.

No pulmonary emboli or deep venous thromboses related to the bypass procedure have occurred. Wound seromas or hematomas in the groin or axilla have developed in six patients. All of these collections resolved spontaneously, two following drainage. During surgery, no changes in blood coagulation profiles attributable to bypass have been seen.

Recent experience reveals that patients with acute Budd-Chiari syndrome are probably not candidates for venous bypass. In two recent cases, attempts at thrombectomy of the clotted portal and vena caval systems followed by establishment of bypass resulted in suboptimal flows in the bypass circuit. In addition, the risk for embolizing preformed thrombus material was recognized to be quite high. Attempts at bypass were abandoned in both of these cases and the anhepatic phase proceeded without incidence.

Other Considerations.

Some of the most important advantages of the venous bypass, however, are not demonstrable by the strict analysis of physiological and survival data. Many situations arise in which portal and caval venous occlusion times must be extended beyond the normal 60 to 90 minutes usually felt acceptable without bypass techniques. This includes any patient in whom the normal, careful, meticulous dissection during the recipient hepatectomy is impossible, and removal of the liver can be accomplished safely only by immediate and complete devascularization of the organ, followed by rapid resection. Hemostasis in the bed is then obtained during the anhepatic phase, and may require an hour or more of intense effort which, if accompanied by increasing portal and caval venous hypertension, may prove fruitless, even dangerous. The bypass system in this situation allows for a period of careful and deliberate hemostasis while keeping the venous beds decompressed and maintaining central venous pressures. It also allows time for careful preparation of vessels for anastomoses, including dissecting out a usable recipient hepatic artery and portal vein if this were not possible before complete hepatectomy. Finally, the bypass removes a great deal of pressure from the surgeon performing the vascular anastomoses since the onus of completing them quickly and yet accurately is somewhat lessened. This offers particular advantages in a training situation.

Future Outlook.

In general, pediatric patients have fared much better than have adults during the anhepatic phase of orthotopic liver grafting. Nevertheless, a method of venous bypass for the small patient would offer distinct advantages in a number of high-risk situations. Application of the apparatus currently in use is hindered by the lower limit of bypass flow (1000 ml/min) required to eliminate the possibility of thrombus formation. In certain situations, the disadvantages of systemic heparinization of the recipient may be overshadowed by the life-sustaining support of a venous bypass. In addition, the laboratory experience that defined the safety of the lower limit of bypass flow was all obtained without the use of heparin-bonded cannulae, tubing, or pump heads. Work is in progress that may redefine the lower limit of safe bypass flow and thus make venous bypass available in pediatric patients.

Last, although the final impact of venous bypass alone has yet to be determined, its routine use has significantly lowered the early mortality which, in the past, has often been associated with a difficult intraoperative course. The fact that its overall impact is not greater underscores the continuing need for better immunosuppression and better definition of the overwhelming risk factors in recipients.

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The multiple-organ procurement procedure was evolved over a period of several years and in its final form was published at the request of the Surgeon General of the United States in the hope of creating a standard approach for excision of many organs from the same donor. This objective has been realized worldwide and has greatly facilitated the transplantation of organs other than the liver.

A flexible procedure for multiple cadaveric organ procurement

Surgery, Gynecology & Obstetrics, 158: 223-30, 1984

Thomas E. Starzl, Thomas R. Hakala, Byers W. Shaw, Jr., Robert L. Hardesty, Thomas J. Rosenthal, Bartley P. Griffith, Shunzaburo Iwatsuki and Henry T. Bahnson

With the improvements in immunosuppression that have occurred in the last few years, a great increase can be expected in the demand for cadaveric kidneys and other vital organs. Doctor Koop, Surgeon General of the United States, has convened conferences and symposia to examine questions of cadaveric organ solicitation, removal, preservation and deployment, In such discussions, it has been obvious that a uniform procedure should be developed which is flexible enough to allow the excision of various organ combinations without jeopardy to any of the individual grafts. This study was done to report the method which we have used with satisfaction for several years.

THE APPROPRIATE DONOR

In the United States, it has become common to remove organs from so-called heart-beating cadavers with pronounced brain death. With some leisure, studies can be obtained in such donors of the function of individual organs. Cardiovascular-respiratory instability may be reflected in the necessity for vasopressor support, poor blood gas values or other adverse findings familiar to intensive care physicians. If such donors are unstable, kidney removal may be all that is possible. However, most brain dead donors can be maintained and improved with conventional intensive therapy and then an attempt can be made to co-ordinate the needs of surgeons who perform transplantations in different parts of the country in light of the availability of potential extrarenal grafts.

THE PRINCIPLE OF CORE COOLING

The intraoperative infusion of cold fluids is common to all preservation techniques. The most important objective is the avoidance of warm ischemia. Cooling of an organ graft by intravascular infusion of chilled lactated Ringer's solution at the time of circulatory arrest was introduced into the laboratory for experimental transplantation of the liver almost a quarter of a century ago (1) and was promptly applied clinically for the preservation of kidneys (2) and all other organs.Such cooling expands many fold the duration of oragan viability and allows the unhurried application, if desired, of other more sophisticated preservation measures. Lactated Ringer's solution has a low potassium content and is nearly isotonic. Chillied special solutions with an electrolyte composition similar to that in cells were shown in a study done in 1969 (3) to extend the permissable limit of cold renal ischemia beyond that achievable with isotonic solutions. The same effect has been shown with livers (4). Cardiac surgeons have cooled the heart with various cardioplegic solutions that have potassium concentrations of about 20 milliequivalents per liter.

The simplest and probably the best way to achieve immediate internal cooling of any organ is by in situ infusion with lactated Ringer's solution. Then, the individual organs may be infused with specified amounts of special solutions after their removeal . The transplantation surgeons in the northeastern part of the United States prefer this approach since it avoids the uncontrolled- infusion of large amounts of the potassium-rich Collins type solutions. However, the use of Collins solution for the in situ infusion of the donor procedure is so widespread that re-education away from this practice has been difficult. Furthermore, the establishment of a uniform code for infusion is not obligatory since the various solutions can be used so easily for the in situ cooling of different organs in the same donor as will be made clear herein.

THE INCISION

A complete midline incision is made from the suprasternal notch to just above the symphysis pubis (Fig. 1). The sternum splitting component of the incision has replaced the cruciate abdominal incision which was commonly used previously. Most hospitals have a bone cutting saw for the sternum, but the Pittsburgh procurement team always carries a Lebsche knife and mallet plus a sternum spreader which have been useful when visiting small hospitals that do not have cardiothoracic equipment. The long incision provides good exposure for removal of the heart, both kidneys, the liver and other thoracoabdominal viscera.

GRAFT NEPHRECTOMY

All of the multiple organ removals can be envisioned as modifications of the evisceration techniques for cadaver kidney removal which have been described in other reports (5, 6).

The extraperitoneal space is entered by incising the peritoneal reflection of the ascending colon, cecum and distal part of the small intestine (Fig. 2). The small and large intestine are swept up and the right ureter is identified, dissected distally and cut across near the bladder (Fig. 2), its tip is tied with a long silk suture, and above this, an incision is made

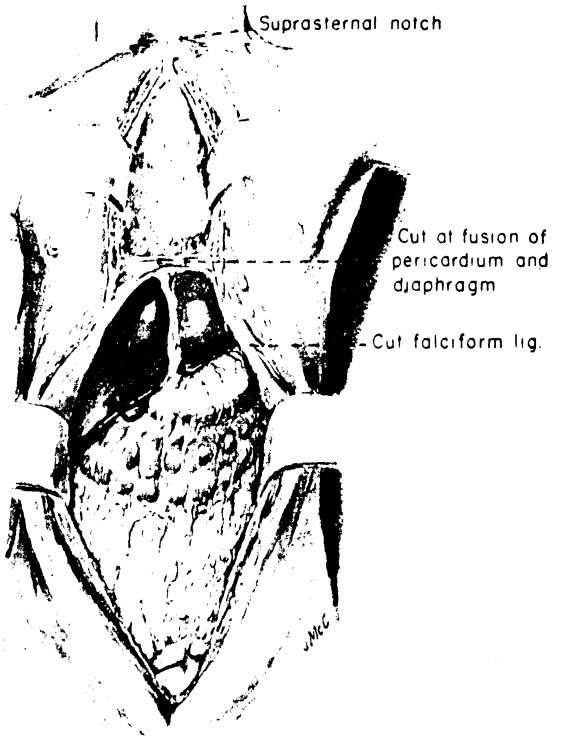

Fig. 1.— Total midline incision used for multiple organ procurement.

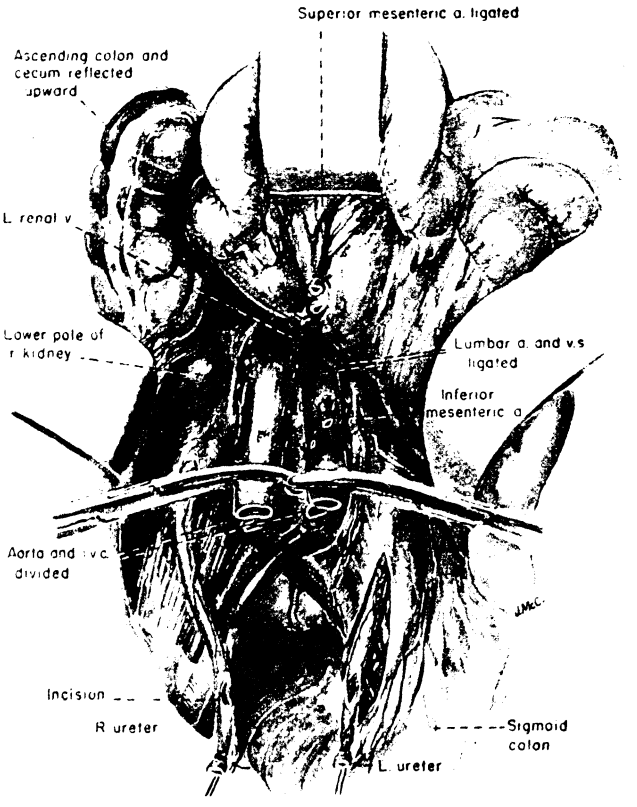

Fig. 2.— Nephrectomy: preliminary steps for in situ infusion of kidneys in a cadaveric donor with an effective circulation who has been pronounced dead as a result of neurologic criteria.

so that urine can escapte. A urine culture may be obtained. The left ureter is treated in the same way either now (Fig. 2) or, if this is inconvenient because of the intact inferior mesenteric vessels, at a later time.

The distal part of the aorta and inferior vena cava are dissected free, encircled and divided (Fig. 2). Both great vessels are mobilized superiorly, ligating and dividing the inferior mesenteric artery and two or three sets of lumbar branches (Fig. 2). Upward dissection of the anterior surface of the aorta is continued until the left renal vein is encountered crossing the aorta from left to right (Fig. 2). Just superior to ths, the superior mesenteric artery can be felt as a taut strand passing almost directly anteriorly providing the intestines are retracted anteriorly and superiorly. The superior mesenteric artery is encircled and ligated (Fig. 2). The viscera are returned to their natural location and attention is directed to a higher level.

The aorta is encircled either just above or just below the diaphragm. The easiest way to have perfect access to the aorta at the more superior level is to deepen the midline incision where the central tendon of the diaphragm fuses with the inferior surface of the pericardium. The fusion is opened up by separating the left part of the tendinous diaphragm from the pericardium, avoiding entry into the pericaridal cavity if possible (Fig. 3). As the dissection proceeds leftward, the left side of the thoracic cavity is encountered immediately and encircled (Fig. 3).

Alternatively, the left triangular ligament of the liver may be incised and the upper part of the abdominal aorta can be encircled just above the origin of the celiac axis (Fig. 3A). This can be done safely after cutting the arcuate ligament and aortic hiatus (Fig. 3). When only the kidneys are to be removed, the celiac axis also should be encircled (Fig. 3A), ligated and divided.

The patient is now given 3 milligrams per kilogram of heparin. Cannulas are placed in the distal part of the aorta and the inferior vena cava (Fig. 4). The clamped aortic cannula is attached to an airfree infusion system through which chilled preservation solutions can later be infused (Fig. 4). The clamped vena cava cannula is attached to tubing that leads to a bleeding bag on the floor. An alpha-blocking agent can be given at this time, and it is a common practice to give drugs, such as chlorpromazine, which are said to stabilize microsomal membranes.

If the kidneys are to be removed, no further dissection is necessary or desirable since infusion of the chilled preservation fluid will be done in situ. Either lactated Ringer's solution or one of the potassium-rich Collins solutions may be used. The aorta is cross clamped just above or below the diaphragm after making sure that the celiac axis and the superior mesenteric artery are ligated or clamped (Fig. 4). The chilled electrolyte solution passes predominantly into the renal arterial supply. The effluent is drained out through the inferior vena caval cannula to the bag on the floor. When in situ infusion of a liter or more in adults has been completed, the aorta is transected at one or the other level previously defined near the diaphragm, and the vena cava is transected above the entrance of the renal veins (Fig. 5A).

The washed out kidneys are now removed from below. The cannulas in the inferior vena cava and aorta as well as the ureters marked with ligatures are gently retracted anteriorly by an assistant who is asked to keep track of these four structures (Fig. 5B). In the meanwhile, the surgeon retracts one of the kidneys anteriorly with a hand placed behind Gerota's fascia and the nurse or another assistant performs the same task on the other side (Fig. 5B). Neither the kidneys nor the four retracted structures are allowed to fall posteriorly at any time. All tissues passing posteriorly are cut, staying close to the ligaments and muscles covering the vertebral bodies (Fig. 5B) and continuing superiorly until the previously transected aorta and inferior vena cava are reached. Once the posterior dissection is completed, the flimsy residual tissues between the gastrointestinal tract and the anterior surface of the specimen are disconnected. The kidneys are taken from the body en bloc and placed in an ice basin on a back table (Fig. 6A). They can be prepared for machine perfusion of the entire specimen if this technique is preferred or the kidneys can be separated for either perfusion of the individual kidneys or preservation in slush. If the kidneys

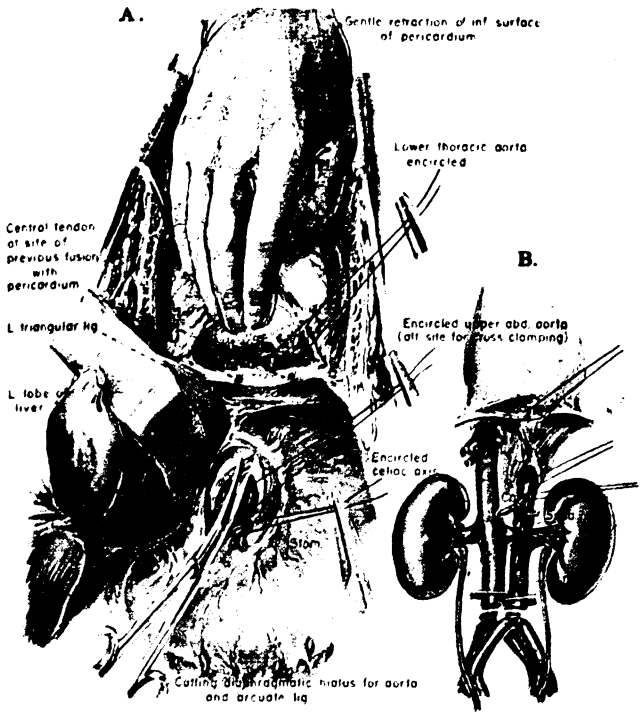

Fig. 3.— A and B, Nephrectomy: preparation for in situ infusion by encircling the aorta just above or alternatively just below the diaphragm.

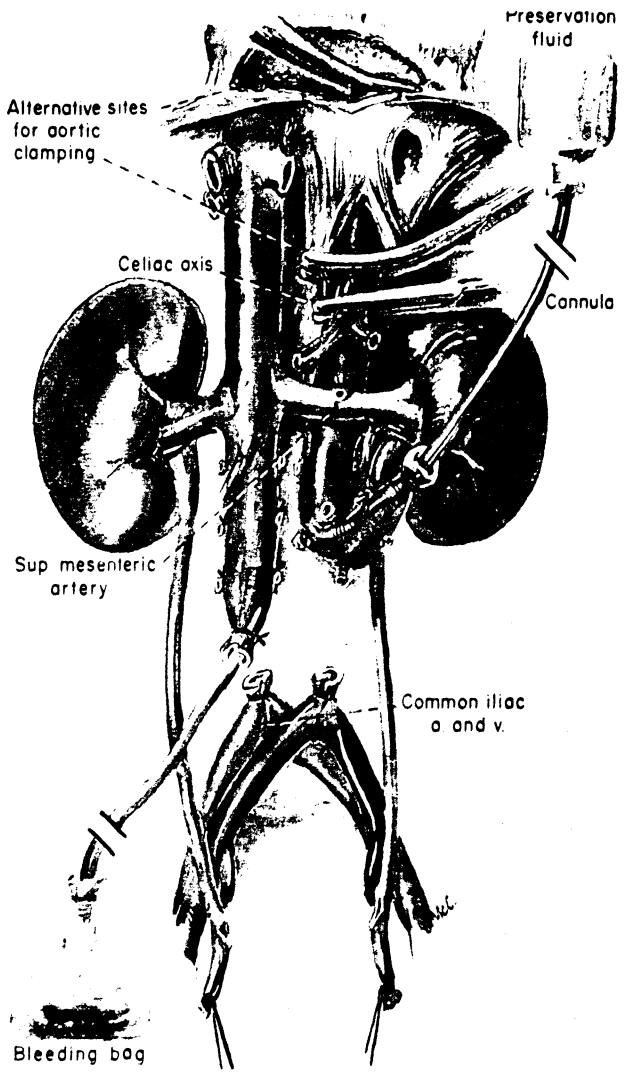

Fig. 4.— Nephrectomy: in situ infusion of kidneys with cold preservation fluid through the distal part of the aorta, with a venous bleed-off from the distal inferior vena cava. The aorta is clamped proximaly at one of the sites shown in Figure 3.

are to be separated, the left renal vein is transected flush at its entranc into the inferior vena cava (Fig. 6B). If slush preservation is planned for kidneys that were cooled by initial in situ infusion with lactated Ringer's solution, a final flush with one of the Collins type solutions is necessary.

For division of the kidneys for slush preservation, it is safest and most convenient to turn the specimen over (Fig. 6B). With one blade of a scissors inserted into its lumen, the posterior wall of the aorta is incised. A perfect guide to the line of aortic incision is the row of ligated or clipped lumbar arteries (Fig. 6B). Then, with a perfect internal view of the renal arterial branches passing laterally, the anterior wall of the aorta is incised longitudinally from the inside (Fig. 6B). If a perfusion is planned, aortic flaps are fashioned during separation and used for closure so that cannulas need not be placed directly into the renal arteries. If the left renal vein was detached from the vena cava as described earlier, it is perfectly safe to divide quickly the remaining structures connecting the right and left kidneys.

After the kidneys or other organs, or both, have been cooled and excised, segments of the iliac arteries and veins are routinely removed (Fig. 7) and placed in a cold tissue culture solution for refrigeration. The thoracic aorta and pulmonary artery may also be taken. Such grafts can be life-saving in the event of unexpected technical problems in the recipient (7).

TOTAL HEPATECTOMY

Removal of the liver requires only minor modifications of the foregoing basic technique. As soon as the midline incision is made, the liver is inspected to be sure that its color and texture are normal. Anomalies are looked for, of which arteries to the left lobe from the left gastric artery or to the right lobe from the superior mesenteric artery are the most frequent. Ways of dealing with such anomalies have been described elsewhere (8, 9) and will not be considered herein.

If the anatomy is normal, the splenic and left gastric arteries are dissected, ligated and divided (Fig. 8), and the celiac axis is dissected as far back toward the aorta as is convenient. The aorta is encircled at one of the locations above the celiac axis as was described in the section on graft nephrectomy (Figs. 3 and 8).

Turning more distally, the gastroduodenal artery and, when present, the right gastric artery are ligated and divided. Beneath the gastroduodenal artery is found the portal vein (Fig. 8). The common bile duct is mobilized to as low as level as possible and transected (Fig. 8). At the same time, the gallbladder is incised and bile is washed out in order to prevent autolysis of the mucosa of the biliary tract. The portal vein is cleaned inferiorly to the junction of the splenic vein and the superior mesenteric vein. The splenic vein is ligated, and through its central end, a cannula is placed through which a slow infusion of lactated Ringer's solution at 5 degrees C. is begun (Fig. 8). The superior mesenteric vein is encircled. If necessary for exposure, the neck of the pancrease should be divided.

Next, the distal part of the abdominal aorta and inferior vena cava are freed and ligated as described in the section on graft nephrectomy (Figs. 2 and 4), but the superior mesenteric artery is not yet tied. Aortic and vena caval cannulas are placed after systemic heparinization. The infusion rate of the cold lactated Ringer's solution through the splenic vein is increased. In adults, the liver can be felt to cool after 1 or 2 liters of infusion at the same time as the body temperature falls. In children, smaller volumes of lactated Ringer's solution are needed for an obvious effect. At this time, the

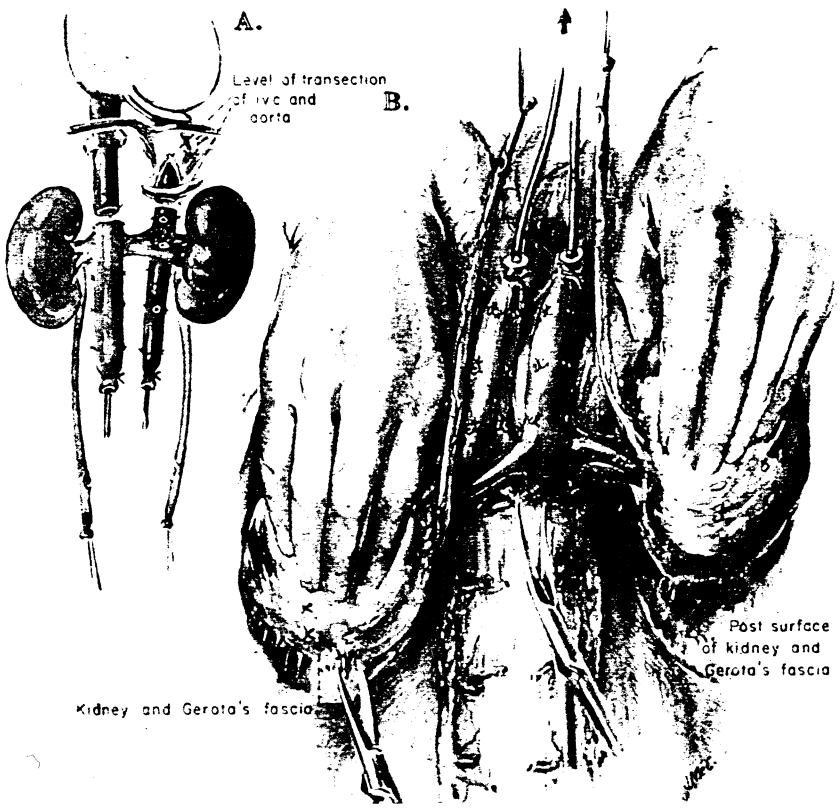

Fig. 5.— A and B, Nephrectomy: removal of perfused kidneys en bloc. The kidneys and great vessels are held anterior to the plane of scissors dissection.

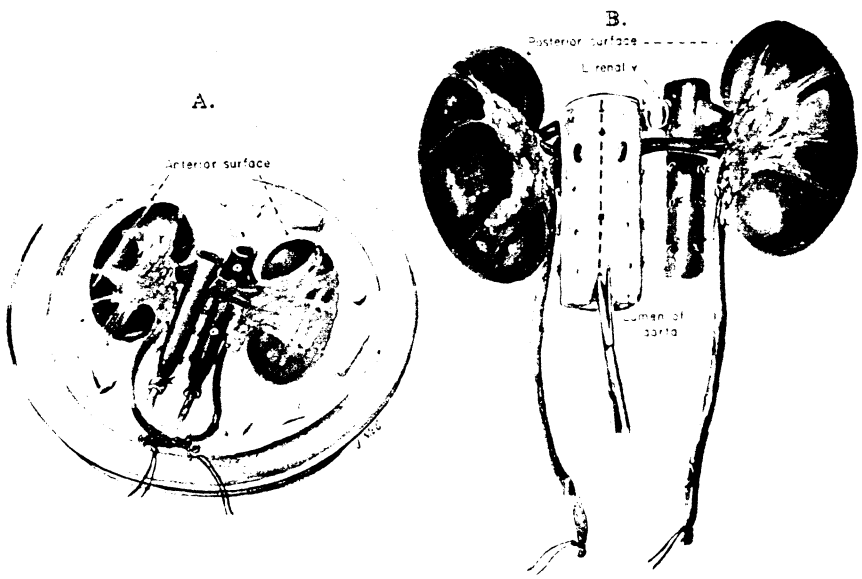

Fig. 6.— A, placement of kidneys in an ice basin. B, Division of kidneys using a posterior approach to split the aorta.

previously encircled superior mesenteric artery (Fig. 8) is tied, followed immediately by ligature of the superior mesenteric vein (Fig. 9). Over infusion is prevented by intermittent bleeding off through the inferior vena cava cannula. The circulation of the donor still intact and the progressively cooling liver still has an hepatic arterial supply. This has been termed the "pre-cooling' phase (9).

Procurement is terminated as with graft nephrectomy by cross clamping the aorta near the diaphragm at one of the sites of its previous encirclement (Fig. 9). Cold solution through the aortic cannula is immediately infused as with graft nephrectomy, and blood from the inferior vena cava is drained onto the floor bag. The right atrium is also opened as an extra precaution against overdistention of the liver. Anesthesiologic support is stopped. Portal infusion with lactated Ringer's solution is discontinued shortly after aortic cross clamping. After 1 to 2 liters of lactated Ringer's or Collins solution have passed in through the aortic cannula, the kidneys as well as the liver are bloodless and cold. The celiac axis is detached from the aorta with an aortic patch or taken in continuity with the full aortic circumference. If further renal cooling is desired, the donor aorta can be clamped just below the celiac axis.

Until this time, dissection of the suprahepatic vena cava has been avoided. Now, the suprahepatic vena cava is dissected free along with the surrounding cuff of diaphragm. The liver is peeled inferiorly cutting posterior attachments, including the right adrenal veins (Figs. 8 and 9). No unusual effort is made to tie individual tributaries to the vena cava at any level since this can be done later at leisure in an ice basin in the recipient operating room after the specimen has been returned to the parent hospital. Since the celiac axis has already been cut free in continuity with a circumferential piece of aorta or an aortic patch, the liver is ready for removal.

The freed liver is taken to a back table, given a final flush in adults with 500 or 600 milliliters of cold Collins type solution and placed in a bag which is filled with the same kind of solution. The bag is sealed and covered with crushed ice in a picnic cooler. The ten or 15 minutes required to remove the liver is not harmful to the kidneys which are not subjected to any warm ischemia whatsoever. With the liver out, removal of the kidneys en bloc is carried out as described in the section on graft nephrectomy. The excision is greatly facilitated by the absence of the liver. Vascular grafts are removed as described previously.

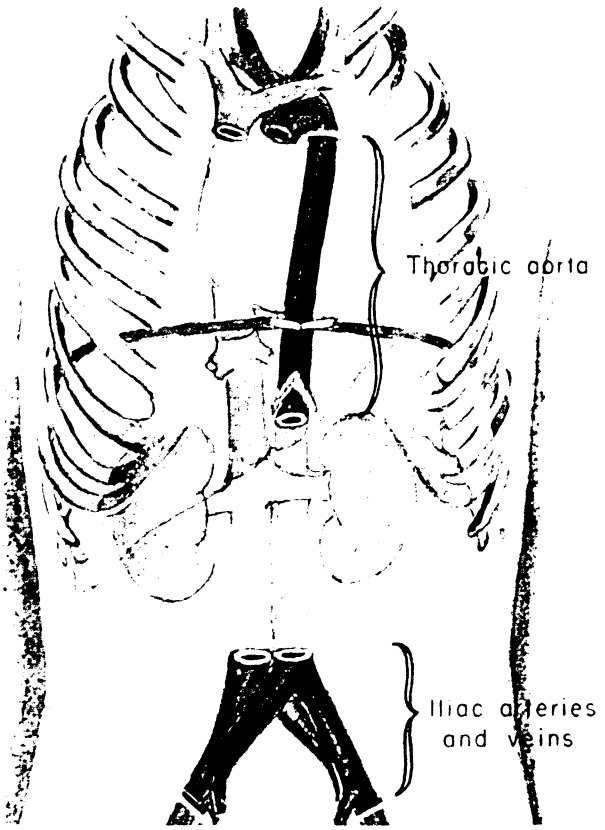

Fig. 7.— Removal of segments of iliac arteries and veins and thoracic aorta. The vascular grafts are refrigerated and kept in case of an emergency.

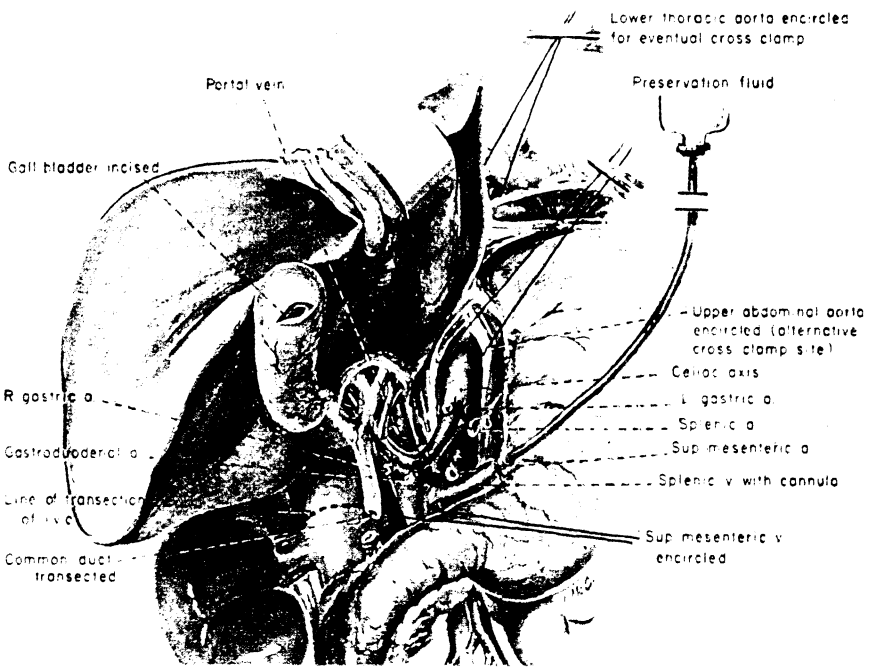

Fig. 8.— Hepatectomy: hilar dissection and transection of the common bile duct as an initial step in multiple organ harvesting. Note that the splenic vein (or alternatively the superior mesenteric vein) is cannulated for eventual delivery of preservation fluid.

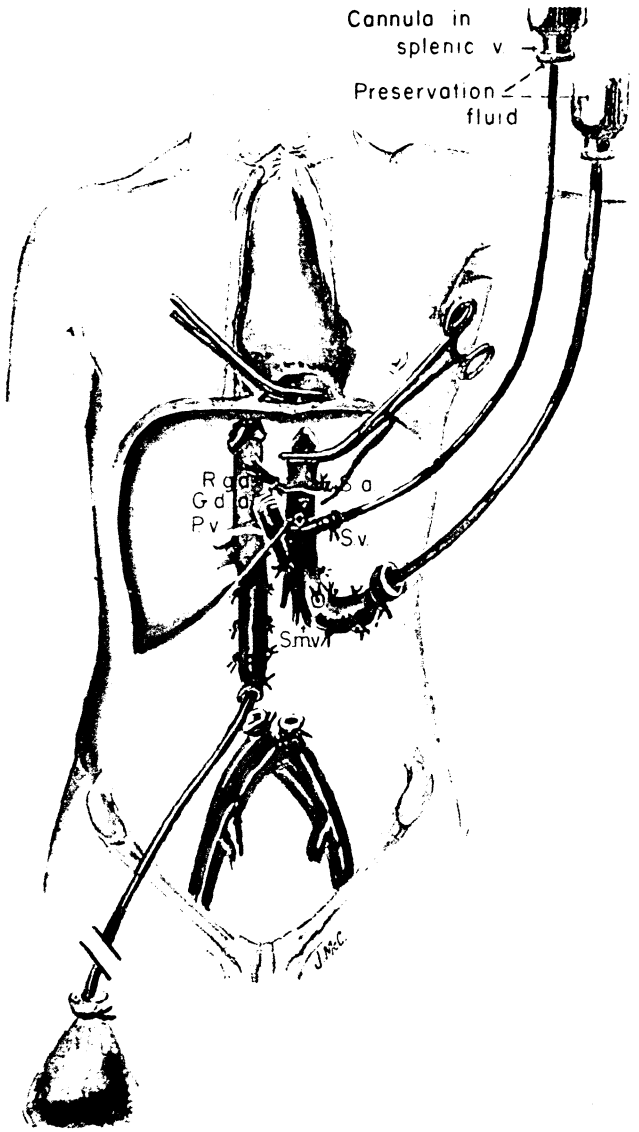

Fig. 9.— In situe infusion technique used when the kidneys and liver are removed from the same donor. *R.g.a.*, Right gastric artery; *G.d.a.*, gastroduodenal artery; *S.a.*, splenic artery; *S.v.*, splenic vein; *P.v.*, portal vein, and *S.m.v.*, Superior mesenteric vein.

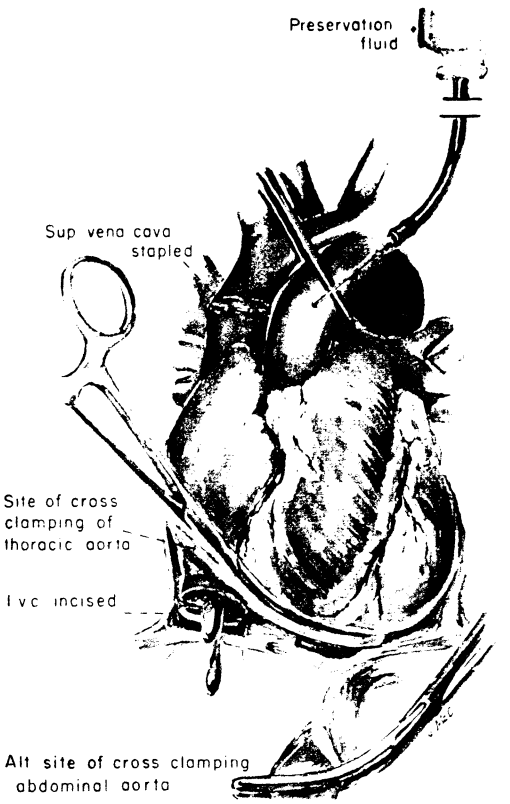

Fig. 10.— Final steps if the heart is to be removed in combination with nephrectomies or hepatectomy, or both. See text for details.

REMOVAL OF THE HEART

With kidneys. The simple preparatory steps described previously are completed. The cardiac team now assumes command. The principle that is followed is to disconnect the thoracic and abdominal aortic circulation by aortic cross clamping at one of the encirclement levels just above or below the diaphragm (Fig. 10) at the precise moment of cessation of heart beat, with immediate graft cooling of both abdominal and thoracic viscera. The preparation for this technical step is the aortic dissection previously described in the section on graft nephrectomy (Fig. 3).

Before this final step is taken, the pericardium is incised, and the aortic root is separted from the main pulmonary artery. The superior vena cava is stapled 2 centimeters proximal to its junction wiht the right atrium, the inferior vena cava is clampled or incised (Fig. 10) and the beating heart is allowed to empty. At the same time as the aorta is cross clamped at the diaphragm, the thoracic aortic arch is clamped at the origin of the innominate artery. Chilled electrolyte solution is infused into both the aortic root (Fig. 10) and into the distal part of the aorta and renal arterial bed (Fig. 4).

The heart is removed first (Fig. 10). Sequentially, the inferior vena cava, left-sided pulmonary veins, pulmonary artery, superior vena cava and right-sided pulmonary veins are divided. The aortic clamp at the level of the innominate artery is removed and the aorta stapled at this level prior to its transection in order to facilitate infusion of the cold cardioplegic solution during implantation of the donor heart. The excised heart is immersed in chilled electrolyte solution during transplantation. After the heart is out, the kidneys are removed as described earlier.

With liver and kidneys. The thoracoabdominal disconnection principle is the same as just described. While the circulation is still intact, the intraportal fusion of cold lactated Ringer's solution is begun into the splenic vein. Should ventricular fibrillation occur as a consequence of infusion the chilled solution, the aorta could be clampled, cardioplegia infused and the heart harvested any time. This foreshortening of the procedure should not be necessary, and with a single exception, the heart always has been removed in our experience under the optimal conditions of liver and kidney cooling. The incidental cooling of the heart by cold blood and fluid coming out of the liver may contribute to smooth cardiac cooling. Care is taken not to remove much of the intrapericardial inferior vena cava since it is not important for the cardiac graft but may be needed by the surgeons performing hepatic operations. After the heart is excised, the liver and kidneys are removed in that order.

EXPENDABILITY FACTROS AND GRAFTS QUALITY

The way in which the organs are removed with multiple graft procurement defines an explicit priority list of heart, liver and kidneys in that order. The heart must function immediately and the liver within a few hours, whereas immediate function of renal grafts is not a prerequisite for recipient survival.

Nevertheless, the rate of acute tubular necrosis requiring hemodialysis in patients in whom kidneys were obtained at multiple organ harvests has been only one-fifth of the lowest incidence reported after the harvest of kidneys alone (10-12). The exceedingly low rate could reflect the acceptance of only very good donors for hearts and livers or the fact that there is a high intensity of skilled surgical and anesthesiologic input in such instances. However, the most important factor probably is the systematic use of a superior method of nephrectomy which totally precludes any period of warm ischemia for the kidneys and which eliminates the manipulation that can unknowingly damage kidneys if renal vascular skeletonization techniques are used.

OTHER ORGAN COMBINATIONS

The general principles herein described can be applied to graft pancreatectomy or intestinal graft removal. If the whole pancreas is transplanted as we recommend, the combination of liver and pancreas removal is incompatible.

SUMMARY

Techniques have been developed which permit removal of the kidneys, liver, heart and other organs from the same donor without jeopardy to any of the individual grafts. The guiding principle is avoidance with all organs of warm ischemia. This is achieved by carefully timed and controlled infusion of cold solutions into anatomic regions, the limits of which are defined by preliminary dissection.

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IV

Part IV
Auxiliary Liver Transplantation and Hepatotrophic Physiology

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Auxiliary Liver Transplantation and Hepatotrophic Physiology

By the time of Welch's first experiments,[1] the concept of transplanting an extra kidney into a heterotopic pelvic site was well accepted. This background in renal transplantation may have influenced those interested in auxiliary liver transplantation. However, this is speculative.

Welch's first mention of auxiliary liver transplantation was a brief note in the *Transplantation Bulletin,*[1] at that time an appendage to the *Journal of Plastic and Reconstructive Surgery.* The following year, Ned Goodrich and Harold Welch (C. Stuart's nephew), two of Welch's residents at Albany Medical College, New York, provided more complete information.[2]

The operation, as originally described by Goodrich and the Welches or slightly modified by later authors, involved the transplantation of an extra canine liver in the right paravertebral gutter or pelvis of a non-related mongrel recipient. The hepatic arterial supply was derived from the aorta or iliac artery. Venous inflow was reconstituted by anastomosing the distal iliac vein or inferior vena cava of the recipient to the homograft portal vein. Welch and Goodrich, and subsequently others, proved that such livers produced bile for several days after transplantation and then ceased to function. The organs had histopathological evidence of rejection, a not unexpected finding since immunosuppressive therapy was not employed.

It was ten years more before auxiliary transplantation was attempted in immunosuppressed canine recipients. A disquieting observation soon was made.[3,4] The auxiliary homografts, inserted by a modification of Welch's techniques into dogs treated with azathioprine, were much more severely damaged than were orthotopically placed livers. The extra organs underwent rapid shrinkage which was easily discernible within two weeks. There was hepatocyte atrophy and fallout with consequent crowding of the intrahepatic portal tracts. These atrophied transplants were incapable of sustaining life. One of the explanations suggested[3] was that the portal venous blood contained some unspecified substance or substances that was important for maintenance of liver integrity.

Marchioro et al[5] provided support for the latter hypothesis in experiments that were designed to divert splanchnic venous flow through the auxiliary liver and away from the recipient's own liver. Now the atrophy affected the native livers but not the homografts. Marchioro remarked:

> Apparently, there is a competition between co-existing livers for some metabolite or other substance in the portal venous blood. That organ which has first access to the portal flow retains its initial functional and morphologic integrity. The other organ, whether it be the homograft or autologous liver, undergoes atrophy predominantly affecting the centrizonular area.

The validity of this general point of view was confirmed by Marchioro in non-transplant experiments,[6] but the nature of the so-called portal hepatotrophic substances remained obscure for several more years.

In 1973, strong circumstantial evidence was uncovered that the most important of the portal hepatrophic substances was insulin,[7] and three years later the hypothesis was tested directly with insulin infusion experiments in dogs submitted to portacaval shunt.[8] It has become accepted universally that substances, especially insulin, in the splanchnic venous blood are important for the anatomic and metabolic integrity of the liver.[9] In addition, it is clear that splanchnic venous blood modulates hepatic regeneration but by mechanisms and to an extent that have not been defined.[10]

Attempts to define the ideal physiologic conditions for auxiliary liver transplantation clarified an important biologic principle and at the same time delineated the requirements for clinical trials with this procedure. The first attempt at auxiliary liver transplantation was made by Absolon et al[11] at the University of Minnesota on 3 November 1964. That child, who suffered from biliary atresia, received the extra liver in the splenic fossa after splenectomy and lived for 13 days before dying of bile peritonitis and sepsis. By early 1968, eight more attempts had been made, all with a fatal outcome (cf. Table I).

The first auxiliary liver transplantation with unquestionable prolongation of life was performed by Fortner et al[12] at the New York Memorial Hospital on 13 December 1972. The recipient, who had biliary atresia, still is alive with a follow-up of 15 years.

At the meeting of the International Transplantation Society in Rome in September, 1978, Fortner was able to collect information on 50 attempts at auxiliary liver transplantation, undoubtedly a minority of the actual number of cases attempted. Fortner's child was the only example of an unqualified success. Subsequently, Houssin et al[14] in France reported 29 months' survival in an adult who was given an extra liver.

With the increased success of orthotopic liver transplantation, interest in the alternative (and as it has turned out, high risk) procedure of providing an extra liver has waned, and very few further efforts have been reported since 1980. The importance of auxiliary liver transplantation's place in history may depend in the the long run on its role in clarifying hepatotrophic physiology.

TABLE 1: AUXILIARY LIVER TRANSPLANTATIONS THROUGH JUNE 1968

Investigator	Date	Disease	Age (Yrs.)	Survival (Days)	Cause of Death
Absolon (11)	11/3/64	Extrahepatic biliary atresia	11/12	13	Bile peritonitis, sepsis
Starzl (15,16)	2/20/65	Alcoholic cirrhosis	50	22	Sepsis, hepatic failure
Starzl (15,16)	7/05/65	Alcoholic cirrhosis	47	34	Sepsis, hepatic failure, GI bleed
Starzl (15,16)	11/3/65	Extrahepatic biliary atresia	1-4/12	0	Cardiac arrest
Cree (17)	10/27/66	Subacute hepatitis	17	2	Hepatic failure
Fonkalsrud (18)	3/02/67	Hepatoma	46	12	Thrombosis hepatic artery, homograft necrosis
Calne (19)	Summer '67	Alcoholic cirrhosis	47	1/2	Hemorrhage
Sheil (20)	4/12/68	Cirrhosis	45	3	Hepatic failure
Starzl (15,16)	6/20/68	Alcoholic cirrhosis	48	24	Sepsis, hepatic & renal failure

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Although Welch previously had published a brief note on auxiliary liver transplantation, this more complete account by the Albany team was a landmark paper in the history of liver transplantation. Goodrich went into private practice in Santa Fe, New Mexico but suffered a stroke at age 50 and retrained in pathology. We were unable to locate him. Harold Welch is Chief of Surgery at Albany VA and Professor of Surgery at Albany Medical College.

C. Stuart Welch died of a myocardial infarction in1980 on his birthday. He was 71 years old. Nelson, then Chief of Surgery at the Albany VA, died of burns from an explosion in his apartment while refinishing furniture. The VA pathologist, Beecher, is retired and is in good health.

Homotransplantation of the canine liver

Surgery, 39: 244-51, 1956

Edward O. Goodrich, Jr., Harold F. Welch, John A. Nelson, Theodore S. Beecher and C. Stuart Welch

In this communication we shall report our experience with homotransplantation of the dog's liver. Our purpose in attempting this transplantation was, first, to find out whether the operation was technically possible with survival of the liver, and, second, to determine the longevity of such transplants and their effect upon the host. The dual blood supply of the liver presents a unique technical problem for which we developed several types of anastomoses. A greater difficulty, however, is the sensitivity of this organ to anoxia.[1] This obstacle is compounded in the dog by the presence of intrahepatic saprophytic clostridia which proliferate rapidly under anaerobic conditions. These organisms cannot be uniformly eliminated although Raffucci and Wangensteen[2] have demonstrated an increased tolerance to interruption of hepatic arterial flow in dogs prepared with wide-spectrum antibiotics.

In the experiments being reported, we attempted to ameliorate the effects of hepatic anoxia during the period of complete interruption of liver blood flow while transplanting the organ by two methods. We first used long-term preparation of the donor with wide-spectrum antibiotics, in an attempt to eliminate the intrahepatic saprophytes. Later, we inserted a polyethylene shunt between the recipient dog's aorta and the hepatic arterial tree of the donor liver while performing the afferent anastomoses. This latter technique proved more satisfactory and will be described in detail.

Experimental Method

Technique of Liver Transplantation Using an Arterial Shunt.— Donor and recipient dogs were prepared simultaneously by two surgical teams, and the entire liver of the donor was transplanted with the accompanying vascular trunks into the lower adbomen of the recipient. The recipient's liver was not disturbed. Donor animals were selected so that they weighed 5 to 10 kilograms less than the recipients. Intravenous Nembutal anesthesia was used and the donor was carried on endotracheal intermittent positive pressure.

By careful dissection the donor's portal vein, infrahepatic and suprahepatic vena cava, and the hepatic artery and its proximal tree were isolated via a thoracoabdominal incision. Since the hepatic artery was usually too small for reliable patency of the anastomosis, it was usually necessary to use the celiac axis or a segment of aorta. With the other branches ligated, the aortic segment provided a readily anastomosed extension of the donor's hepatic artery. Before removing the liver, the donor was heparinized.

At the same time, the recipient's vessels were prepared through a lower right rectus abdominal incision. The infrarenal inferior vena cava, the distal aorta, and external iliac arteries were isolated. The vena cava was transected and fixed over a Blakemore-Lord cuff,[3] in preparation for nonsuture anastomosis to the subcardiac vena cava of the donor. The site of transection of the aorta was then elected, and a polyethylene tube was inserted into this vessel in a position proximal to the level of transection.

When the recipient had been prepared, the donor's vessels were transected as rapidly as possible, in the following order: the infrahepatic vena cava which was ligated, the portal vein, the aorta below and above the celiac axis, and the subcardiac vena cava. The liver was then placed in the lower abdomen of the recipient and the hepatic outflow was first established by completing the vena cava anastomosis over the previously placed Blakemore-Lord cuff. The polyethylene shunt was then connected to another tube placed in a branch of the hepatic arterial tree of the donor liver. When using the aortic segment preparation, the superior mesenteric artery proved to be the most suitable location for the shunt. With the shunt in operation, it was then possible to perform both proximal and distal aortic anastomoses accurately and less hurriedly, meanwhile being assured of adequate oxygenation of the transplanted liver. When the arterial anastomoses were completed, the shunt was discontinued and the portal vein of the donor was connected to the recipient's distal vena cava over a second Blakemore-Lord cuff. To prevent motion of the transplant and kinking of the suprahepatic vena cava, the diaphragmatic tags on the suprahepatic vena cava were tacked to the right psoas fascia with one or two sutures. Finally, a plastic cholecystostomy cannula* was inserted and brought out through the wound, which was then closed in layers. Fig. 1 is a diagrammatic representation of the liver transplant with anastomoses.

Liver biopsies were obtained before and after transplantation and at daily intervals after operation. Bile output was recorded and in some animals daily Bronmsulphalein extraction curves were obtained. The

* Manufactured by Mr. L. W. Carson, Wheaton, Ill.

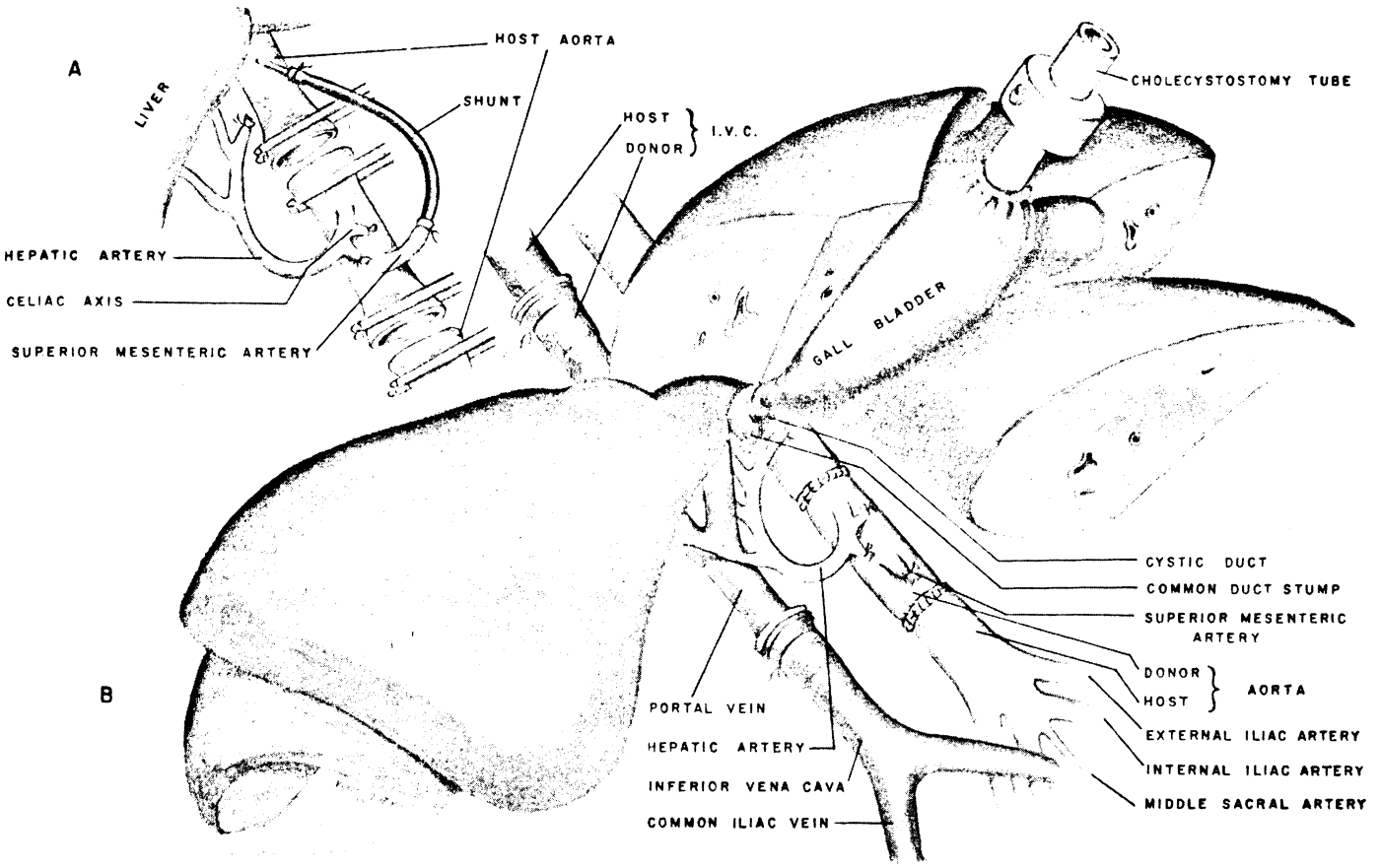

Fig. 1.— A, Demonstrates the temporary shunt between the recipient dog's aorta and the donor liver. The course of arterial blood flow from the recipent's aorta is via the polyethylene shunt to the superior mesenteric artery and aortic segment of the transplant. B, Demonstrates the completed liver transplant and shows the afferent and efferent anastomoses as described in the text.

clinical condition of the animals was closely followed and antibiotics, fluids, and blood were given as indicated. Upon death or sacrifice post-mortem examinations were performed.

Other Techniques for Liver Transplantation.— The technique described here was developed after a number of other methods had proved less satisfactory. Arterialization of the donor's portal vein by the recipient's external iliac artery, after the method of Schilling, was first used.[4] In these experiments the hepatic arterial tree was not used. Later, end-to-end anastomosis of the recipient's external iliac artery to the donor's hepatic artery or celiac axis was combined with anastomoses of a recipient common iliac vein to the donor portal vein. All of the animals in the earlier experiments were prepared with oral wide-spectrum antibiotics† for periods ranging between four and twenty-one days, in an attempt to prolong the hepatic tolerance to anoxia. No shunt was used in these experiments. Animals in this group were followed postoperatively in the manner outlined previously.

Results

Survival of Transplants.— Homotransplantation of the canine liver has been performed successfully twenty-one times in forty-nine attempts. In all successful cases the animals lived at least five days, and in no unsuccessful experiment did the recipient survive over thirty-six hours. In all successful experiments, bile was produced for from one to seven days with one exception, in which viable hepatic tissue was demonstrated by needle biopsy on the first postoperative day. In five of the animals having successful transplants, removal of the recipient's liver was performed on one of the days following transplant. None of the recipients survived this procedure. The shunt technique as described was used in thirteen pairs of animals with 69 per cent successful transplantations, while in thirty-six pairs of animals prepared with wide-spectrum antibiotics and having various types of afferent anastomoses there was 33 per cent survival.

Typically, in a successful transplant experiment, bile began to rise in the cannula as the wound was being closed. The animals recovered from the anesthesia within one to four hours, but remained lethargic for from twelve to twenty-four hours. During the first day bile was produced at a rate of 2 c.c. per kilogram of donor body weight. Solid food was occasionally taken at this time, but more frequently the diet was voluntarily limited to liquids. The temperature and pulse rate were normal or slightly elevated, but no local signs of peritoneal irritation were manifested when omentum was draped over the liver prior to closure. By the end of the second day, bile output increased, and the animal appeared to be approaching a more nearly normal state. During the third twenty-four-hour period, bile secretion by the transplant was usually maximum and amounted to 4 to 5 c.c. per kilogram of donor weight. During this period the bile was very dark, mucoid, and rather typical of gall-bladder bile. In the subsequent two days, the bile output fell off and the bile became less intensely colored and more watery. About the fifth day secretion ceased and was followed in one or two days by a serosanguineous, then a foul sanguino-purulent discharge via the cholecystostomy cannula. After the fifth day survivors exhibited varying degrees of toxemia which were frequently fatal, and exploration after cessation of the bile flow revealed a completely necrotic liver, usually infected, in the process of degeneration and sloughing.

† The chlortetracyclene and tetracyclene used in these experiments were supplied by Lederle Laboratories, Division of American Cyanimid Company.

Microscopic Studies of Liver Biopsies.— The microscopic picture of the transplanted liver usually correlated well with the bile output, although occasional inconsistencies were found. These were thought to be due to failure of some portions of the transplant to receive an adequate blood supply while other portions of the organ were functioning. Liver biopsy taken immediately after transplantation showed morphologic changes of varying intensity. In practically every instance there was some sinusoidal congestion which seemed to extend peripherally from the region of the central vein, and in extreme cases this congestion caused disruption of the hepatic cords. Where frank necrosis of individual hepatic cells occurred, it was usually centrolobular and was accompanied by a polymorphonuclear leukocytic infiltrate. When the experiment terminated within thirty-six hours, a wide range of microscopic changes were found, ranging from a slight progression of these changes to massive necrosis with bacterial invasion and intense acute inflammatory exudate. Both needle biopsies and sections of successful transplants taken at exploration or autopsy showed a patchy distribution of lesions. Serial biopsies, therefore, sometimes exhibited rather marked differences, with relatively badly damaged tissue being followed the next day by a specimen exhibiting relatively minor change. When viewed as a group, however, the pattern of change was fairly constant. In some of the surviving animals slight recovery from the congestion and cellular changes seen immediately postoperatively was found. In no instance, however, did the liver ever regain its control status. In a few instances focal anemic necrosis associated with congestion and hemorrhages with acute inflammation progressed rapidly so that the liver was more or less totally destroyed by the second or third day.

Usually, however, beginning on the fourth day, there was more or less general increase in cellularity of the portal spaces. The exudate consisted of numbers of small round cells of the chronic inflammatory type and larger mononuclear cells, presumably fibroblasts. This reaction continued in a progressive fashion. At about the same time, rather extensive necrosis of the remaining hepatic tissue occurred. This was usually patchy in distribution but eventually became generalized. In the end, the liver was totally necrotic and all anatomic features were lost. In only one animal was any viable tissue seen after the sixth postoperative day. In this particular instance, moderate amounts of apparently viable tissue were found about the periphery of the lobules, whereas the central portion showed extensive anemic necrosis, congestion, and acute inflammatory infiltrate. This animal died of distemper on the seventh day.

Bromsulphalein Extraction.— Bromsulphalein extraction curves obtained on five survivors also correlated with bile production and the microscopic picture, indicating maximal transplant function on the third day. In Fig. 2, extraction curves for Bromsulphalein taken in one experiment are shown.

Complications.— Occlusion of the venous return from the hindquarters in the early experiments caused edema in several animals, and the development of abdominal collaterals increased the hazard of needle biopsy. Therefore, direction of the venous return from the legs through a portal vein of the donor liver was effected in perfecting the transplantation technique. A few cases of right hindlimb paralysis were precipitated by interruption of the right external iliac artery, but this complication did not develop in those animals in which the distal circulation was re-established.

Employment of the shunt technique doubled our survival rate and reduced the necessity for completing the arterial anastomosis hurriedly, thereby reducing the incidence of thrombosis. It also obviated the labor and expense of prolonged preoperative preparation of the donor in an uncertain attempt to render the liver bacteriologically inactive.[5] The shunt was found to require constant attention during anastomosis. On several occasions the vessels of the transplant became twisted about the end of the relatively rigid shunt, causing premature thrombosis within the tube, and quite possibly within the artery, also.

Of the twenty-eight transplantations which were failures, eight were

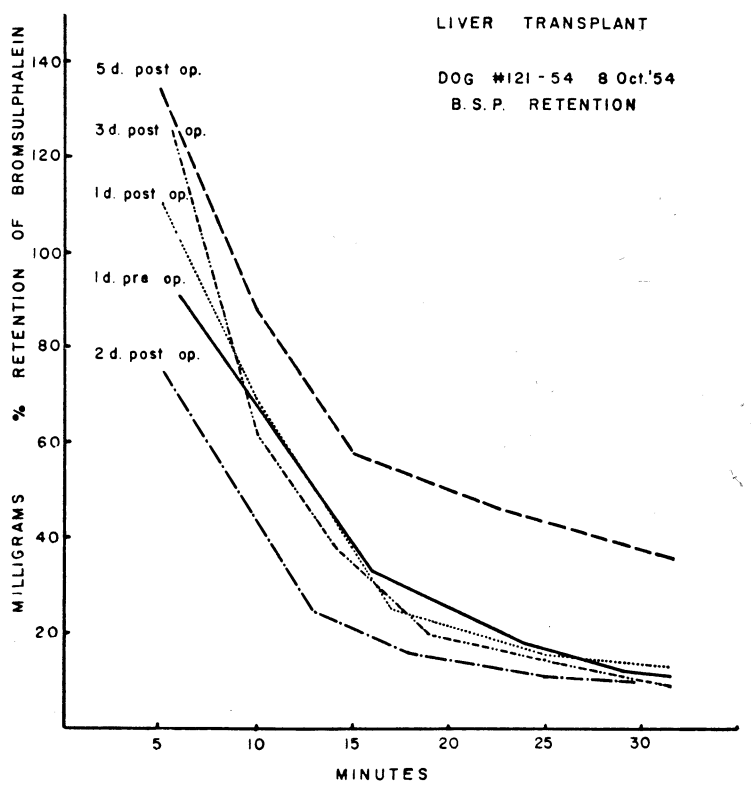

Fig. 2.— The values for Bromsulphalein retention in the serum of a dog in whom a liver transplant is functioning are shown above. Five milligrams per kilogram of the combined weights of both dogs (recipient and donor) was given. The curve designated 1 d. preop. is the control. Curves on the second and third postoperative days show that a more rapid extraction of the dye occurred, the result of the additive function of the liver transplant. On the fifth day dye extraction was less than normal. At this time the transplant was nonfunctioning.

due to what can be called, for lack of a better term, "toxemia."[6,7] In six of these the liver was completely anoxic for over thirty minutes, and in three gross gas bubbles were encountered in the liver at post mortem. In no case did the recipient survive when the donor liver was anoxic for over thirty-three minutes. Afferent arterial anastomosis thrombosis caused eight failures when arteries smaller than the aorta were used, although one liver secreted 15 c.c. of bile for six hours before death. Intraperitoneal hemorrhage caused four deaths. In two instances the efferent vena caval anastomosis was twisted, causing multiple fractures in the dome of the liver, while in one case a dehiscence occurred at the portal iliac vein cuff anastomosis. No source of hemorrhage was established in the other animal. Aspiration of gastric contents caused one death, and in one experiment the animal died of a massive pulmonary embolism after release of the vena caval clamp. The occluding clamp had been placed about 3 cm. below the highest point of dissection on the vena cava, contrary to our usual practice, and had thereby allowed a thrombus to form between the clamp and the renal vein. No cause of death could be determined in five cases.

Discussion

Homotransplants of kidneys in animals have been shown to survive for from one to eighteen days, with the majority of investigators reporting an average of four days of transplant function.[8] These organs are then destroyed by the inevitable host reaction which is thought to be an immunologic phenomenon.[9] We have been able to demonstrate that the liver can be transplanted with maintenance of viability for varying periods up to one week. It would appear, therefore, that the canine liver is subject to the same forces which govern the course of renal homotransplants in this species. Of great significance is the finding of Hume and associates[8] that homotransplants of kidney may survive much longer periods in the human being than in the dog.

It is quite improbable that human livers could be transplanted with survival of the transplants, not so much because of the technical difficulties but more so because of the difficulty of obtaining a suitable donor liver. There is no question but that a temporary functioning liver transplant would have a place in the treatment of patients with chronic liver disease and especially those in acute failure or coma. In the latter condition recovery of the liver is possible in many instances and a temporarily functionning transplant would tide the patient over. It is our present interest to determine how effective liver transplants can be in animals with hepatic failure and deficiency states. Short of actual transplantation of the liver is the technique of viviperfusion using animals in liver failure and donor livers. This technique is being investigated currently in our laboratory. The subject of heterotransplants in the case of the liver is also of interest in that short-term function of donor livers would have value.

The peculiar bacterial flora of the dog's liver[10] and its uncertain and difficult eradication[11] caused a significant number of failures in the group prepared with antibiotics, since there was no way to predetermine the efficacy of our preparation. This factor was controlled in the present experiment by reducing the period of complete hepatic anoxia to a few minutes, through the use of an arterial shunt. Since the human liver is usually sterile, we believe that it is of importance to investigate the reactions which occur after transplantation of normally sterile livers in other species. We have transplanted livers in monkeys (*Macacus rhesus*) but, so far, the technical difficulties have not been overcome and we have had no survivals.

Another difficulty encountered in this study in which dogs have been used, namely, central lobular congestion, may be due to the activity of the hepatic vein sphincters.[12]

At the present writing we have very little data on the composition of the bile excreted by the liver transplants. The active formation and excretion of bile is incontrovertible and the gross appearance and texture of this bile is quite normal. We have noted that during periods of greatest transplant activity, as indicated by Bromsulphalein retention curves, definite blue discoloration of the bile occurred for one to two hours after injection of the dye.

Liver transplants may prove useful in the study of hepatic physiology. Although technically difficult and time-consuming, the procedure may also provide a useful technique for investigations in such varied fields as shock, homeostasis, immunity, and hormone metabolism.

Summary and Conclusions

A description of several techniques used for the transplantation of the liver in dogs has been given, together with observations on forty-nine experiments.

The entire liver of the dog can be transplanted intact to another dog with preservation of function in so far as the excretion of bile is concerned for periods up to five days.

The liver seems to be subject to the same type of homotransplantation reaction as is the kidney when transplanted in the dog. Microscopically, the changes first seen in the liver are small round cell and mononuclear infiltration of the periportal spaces which occur about the fourth day. Thereafter, necrosis begins and progresses rapidly, being complete usually by the end of the fifth day.

Bile production and Bromsulphalein extraction are maximum on the third day after transplantation of the liver.

None of the canine livers in this experiment tolerated complete anoxia for a period over thirty-three minutes, in spite of preparation with antibiotics.

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As soon as immunosuppression was used to prolong the viability of auxiliary liver grafts, it was noted that the extra organs underwent profound atrophy and were damaged far more than livers placed orthotopically (cf. Part I, Starzl, T.E. et al, *Ann Surg,* 1964). The experiments by Marchioro provided evidence that there were specific substances in the portal venous blood without which an optimum environment for a liver graft could not be provided.

Marchioro is Professor of Surgery at the University of Washington, Seattle. In this and other studies establishing the hepatotrophic concept, the impeccable work of Porter, the pathologist, always provided the principal evidence. Porter is Professor in the Department of Pathology, St. Mary's Hospital and Medical School, London.

Physiologic requirements for auxiliary liver homotransplantation

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T. L. Marchioro, K. A. Porter, T. C. Dickinson, T. D. Faris and T. E. Starzl

For hepatic homotransplantation, two general types of operative procedures are available. One, which would be applicable for the treatment of malignant disease of the liver, consists of removal of the recipient's own organ and replacement with a hepatic homograft. The other variety of operation, which was first described in principle by Welch and Goodrich and their associates (Fig. 1A), is designed for the treatment of patients with hepatic failure due to benign disease, such as cirrhosis, and does not involve recipient hepatectomy.

There would be important advantages in performing hepatic homotransplantation without recipient hepatectomy. The formidable task of extirpation of a liver would be avoided. In addition, any remaining function of the diseased host liver would be retained so that immediate total dependence upon the homograft would not be necessary. In spite of these appealing features, the disconcerting observation was recently made[12,13] that there was very marked and rapid reduction of the size of auxiliary homografts which were placed by Welch's technique into dogs receiving immunosuppressive agents, the diminution of homograft mass beginning within 2 weeks. Different explanations for this phenomenon were suggested, including the possibility that there was competition for nutritional substrate by the two livers with the result that the dog's own organ, which received the alimentary venous flow first, was operating at a physiological advantage in comparison to the ectopically placed homograft.

In the present study, the role of portal venous flow in affecting the well-being of the homograft and the host's own liver was studied by a series of experiments designed to allow one or the other liver to have primary access to the splanchnic venous flow. The consequence of the study has been to clarify those physiologic factors which appear to be requisite for the successful employment of auxiliary liver homografts.

Methods.

Twenty-six mongrel dogs, weighing 11.3 to 24.1 kilograms, were used for recipients. The experiment was carried to completion in 15 of these animals, and the group constitutes the basis for the present report. Liver homografts were obtained from healthy animals weighing from 0.5 to 5 kilograms less than the recipient. All operative procedures were performed under pentobarbital anesthesia.

Each recipient animal was treated with azathioprine, 2 to 8 milligrams per kilogram per day, as the sole immunosuppressive agent. This drug was administered orally except during the immediate postoperative periods, at which time the intravenous route was used. As soon as possible the animals were returned to standard kennel feedings. In 3 of the dogs, removal of their own livers was carried out 61 to 77 days after homotransplantation, by a method which preserves the intrahepatic inferior vena cava.[12] For these dogs, azathioprine was continued; and, in addition, radioactive sulfur methionine was administered intravenously to one dog every 5 days after hepatectomy, each dose containing 88 microcuries of radioactive sulfur in approximately 1.8 milligrams of methionine. No continuous infusions of glucose were used after hepatectomy and the preexisting high protein diet was resumed as quickly as possible.

Postoperative hematologic studies were obtained daily in order to determine the safe dose of azathioprine. Biochemical determinations were performed twice a week. These consisted of the measurement of serum bilirubin by the Malloy-Evelyn technique, alkaline phosphatase by the Bodansky method, serum glutamic oxalacetic transaminase and serum glutamic pyruvic transaminase in Sigma-Frankel units, and fibrinogen analysis using Ratnoff's method. In addition, blood sugar, blood urea nitrogen, stool color, fluid intake and output, and daily weights and temperatures were recorded.

At varying intervals postoperatively the animals were re-explored. Biopsy specimens were obtained from the host livers and from the homografts, and estimates were made concerning the relative sizes of the 2 livers. In addition, operative angiograms were performed in the majority of animals in order to demonstrate the patency of the anastomoses and to obtain general information about the direction and magnitude of venous blood flow. The latter studies were performed with 50 per cent hypaque, by injection into the appropriate portions of the arterial and venous systems. In addition to the pathologic studies made by open biopsies, complete autopsies were obtained in each instance as quickly as possible after death. All extraneous tissue was dissected free from both livers, and comparative weights of the homograft and the dog's own liver were obtained. The patency of each anastomosis was specifically noted. Tissues were fixed in formol-saline and Carnoy's solution for histologic studies. Sections were routinely stained with hematoxylin and eosin; Gordon and Sweet's silver impregnation for reticulin; Verhoeff and van Gieson's

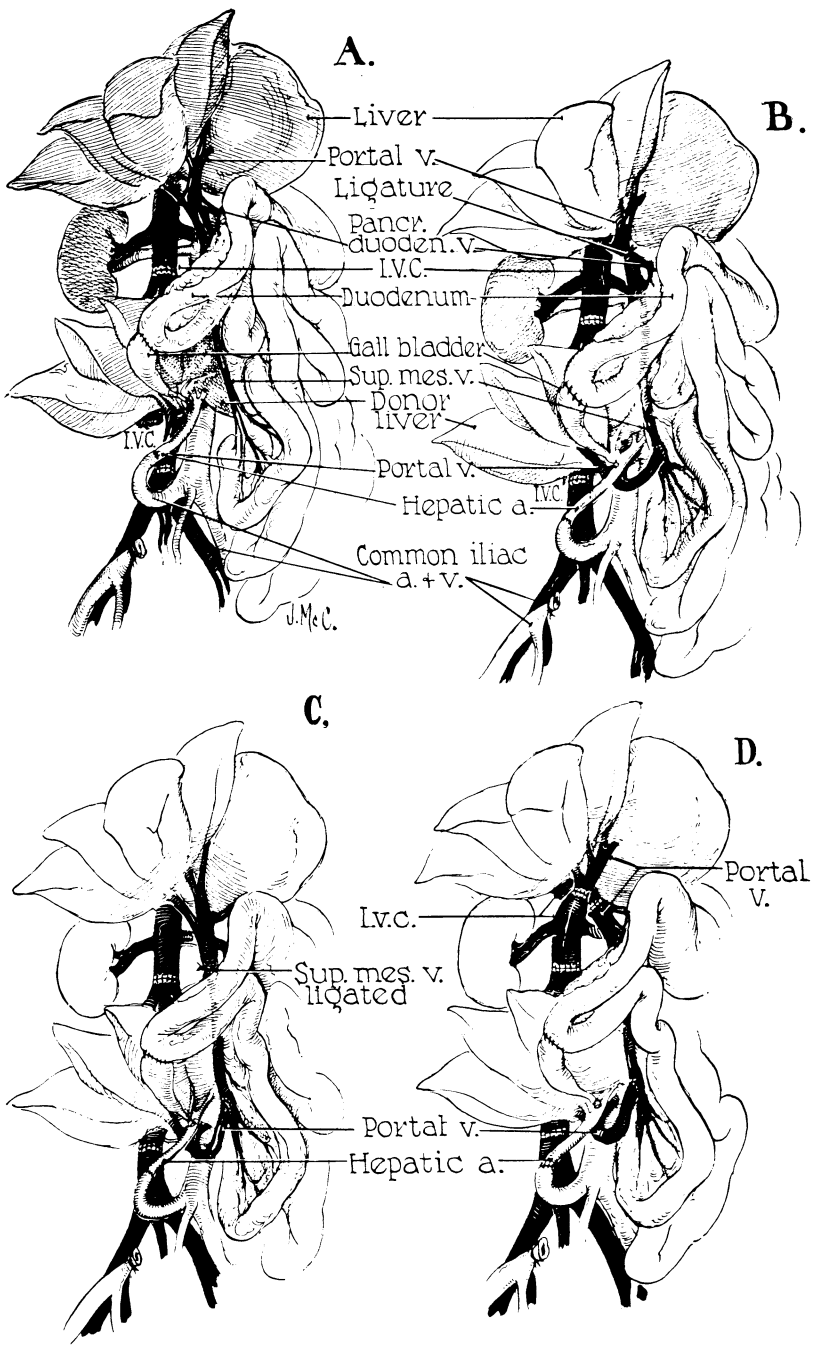

Fig. 1.— Experimental protocols for auxiliary hepatic homotransplantation. A, Previously reported modification of Welch-Goodrich hepatic homotransplatnation. Homograft undergoes rapid atrophy and diminution in size. Portal blood flow is from the systemic venous system. B, Preparation of group 1 dogs in present study. Nonhepatic splanchnic flow is diverted through the homograft. With this preparation, the homograft retains its size and the animal's own liver undergoes shrinkage. It is usually more convenient to bring the hepatic artery behind, rather than in front of, the portal vein as depicted. C, Superior mesenteric vein is ligated below the splenic vein, partitioning nonhepatic splanchnic flow between the autologous liver and the homograft. Homograft shrinkage occurs but more variably (group 2). D, Auxiliary homotransplantation in group 3. The host liver vascularized as with the portacaval transposition of Child.

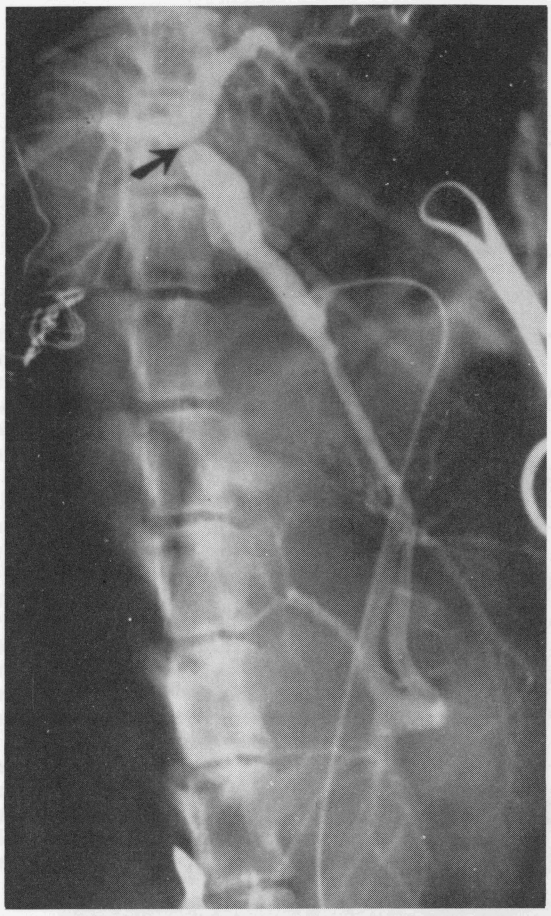

Fig. 2.— Operative venous angiogram of dog 5 showing retrograde passage of nonhepatic splanchnic flow through the homotransplanted liver (lower). The ligature around the portal vein is evident (arrow). The dog's own portal system has also filled with dye from collaterals. Note the large size of th homograft compared to the small dimensions of the dog's own liver.

method for elastic and connective tissue; periodic acid Schiff; Perl's Prussian blue method for iron; and Kutlik's bile stain. Other special stains were used if appropriate.

Three different types of auxiliary homotransplantation were performed:

Group 1. Six homografts were placed in the right paravertebral gutter. The donor vena cava was interposed into the recipient terminal inferior vena cava (Fig. 1B). The hepatic artery was revascularized by means of an end-to-end anastomosis with the right common iliac artery. The end of the homograft portal vein was anastomosed to the side of the recipient's superior mesenteric vein (Fig 1B), using No. 6-0 silk. The recipient's own portal vein was ligated above the most cephalad tributary, the pancreaticoduodenal vein (Fig. 1B). The splanchnic blood flow was thus directed in
a retrograde fashion into the homograft, so that the dog's own liver was supplied only by the hepatic artery.

Group 2. In 6 dogs, a similar operative procedure was carried out except that the ligature was placed on the superior mesenteric vein just below its junction with the splenic vein (Fig. 1C). The venous return from the small intestine was thus diverted through the homograft and the venous flow from the splenic and pancreaticoduodenal areas passed through the recipient animal's own liver. There was consequently a division of the nonhepatic splanchnic flow, both livers receiving a fraction.

Group 3. In 3 animals portacaval transposition was performed from 3 to 9 months before definitive transplantation. After complete recovery from this preliminary operation, an auxiliary homograft was placed in the same location as in groups 1 and 2 (Fig. 1D). The portal vein was then ligated above the entrance of its highest tributary. This modification was designed to give both livers a complete arterial and venous blood supply. The nonhepatic splanchnic venous flow first passed through the homograft and then via the cavaportal anastomosis to the recipient's own liver.

Results in group 1.

Survival. Six of the 10 animals survived operation. In 3 dogs both livers were in place for 25, 28, and 52 days, after which the dogs died of pneumonia, hemorrhage from a cecal ulcer, and from homograft necrosis, respectively. In the first 2 instances, all vascular channels were found to be patent at autopsy. In the third, the vessels were proved to be patent at angiography 30 days after homotransplantation, but in the subsequent 22 day interval the portal-mesenteric anastomosis clotted. The other 3 dogs were subjected to second stage complete removal of their own livers.

Survival after recipient hepatectomy. Three dogs underwent removal of their own livers (Table I) after 77, 61, and 73 days. At the time of hepatectomy an estimate was made of relative masses of the 2 livers, and in each instance the homograft was definitely larger than the animal's own liver, which had undergone striking shrinkage. The first of these, dog 5, resumed a full diet within 2 days and was in excellent clinical condition for the remainder of his life. He was re-explored for open hepatic biopsy 43 days later, and 6 days after this procedure was found dead in his cage from a complete evisceration that had occurred during the night.

The second, dog 8, also recovered promptly from pentobarbital anesthesia but died 8 days later from the consequences of wound dihiscence, peritonitis, and massive gastrointestinal hemorrhage from an acute duodenal ulcer.

The third, dog 10, also recovered promptly from hepatectomy, but his subsequent course was complicated by massive ascites. Twenty-seven days after hepatectomy, he was re-explored for biopsy and died the following day.

After hepatectomy, it was noted that the appetite and nutritional requirements for the 2 longest surviving dogs were markedly increased, as much as 5 or 6 times the ordinary diet being consumed daily, despite which a weight gain did not occur.

Angiographic studies. In 3 animals, dogs 5, 8, and 9, the blood supply to the host's own liver and the homograft was investigated by injection of a contrast medium into the terminal aorta and the splenic vein. In each, a major portion of the nonhepatic splanchnic venous flow appeared to pass through the homograft, although dye was noted to enter the host liver through collaterals (Fig. 2). All arterial anastomoses investigated were found to be open. In 1 animal in which the portal-mesenteric venous anastomosis was found to be patent at angiography, subsequent thrombosis was found at autopsy 22 days later.

Biochemical studies. Following placement of the auxiliary homograft there was a prompt rise in alkaline phosphatase, beginning within a few days after operation (Fig. 3). The serum glutamic oxalacetic transaminase and serum glutamic pyruvic transaminase followed a similar but variable pattern. Chemical jaundice was observed in only 1 dog, the highest value being 1.9 milligrams per cent. Fibrinogen tended to rise early in the postoperative course as has been previously described[12] with the Welch-Goodrich preparation.

Following removal of the animal's own liver, sharp increase in bilirubin was noted in all 3 dogs during the first few days of total dependence upon the homograft, to levels as high as 14.5 milligrams per cent (Fig. 3). The stools became temporarily clay-colored and the urine deepened in color. Despite this, these animals appeared to be in good general condition, and within 1 week the jaundice began to recede in all (Fig. 3), stabilizing between 4 and 7.6 milligrams per cent at the same time that bile reappeared in the stools. Hypoglycemia was not observed. Rises of alkaline phosphatase, serum glutamic oxalacetic transaminase and serum glutamic pyruvic transaminase occurred after autologous hepatectomy with subsequent partial return toward, but never to, normal (Fig. 3). The stools of these animals frequently were observed to contain poorly digested food, the excreta resembling the dog food which had been recently ingested. Stools wre voluminous. Analysis of the diet and stool for fat demonstrated almost complete lack of fat absorption in the 2 longest survivors.

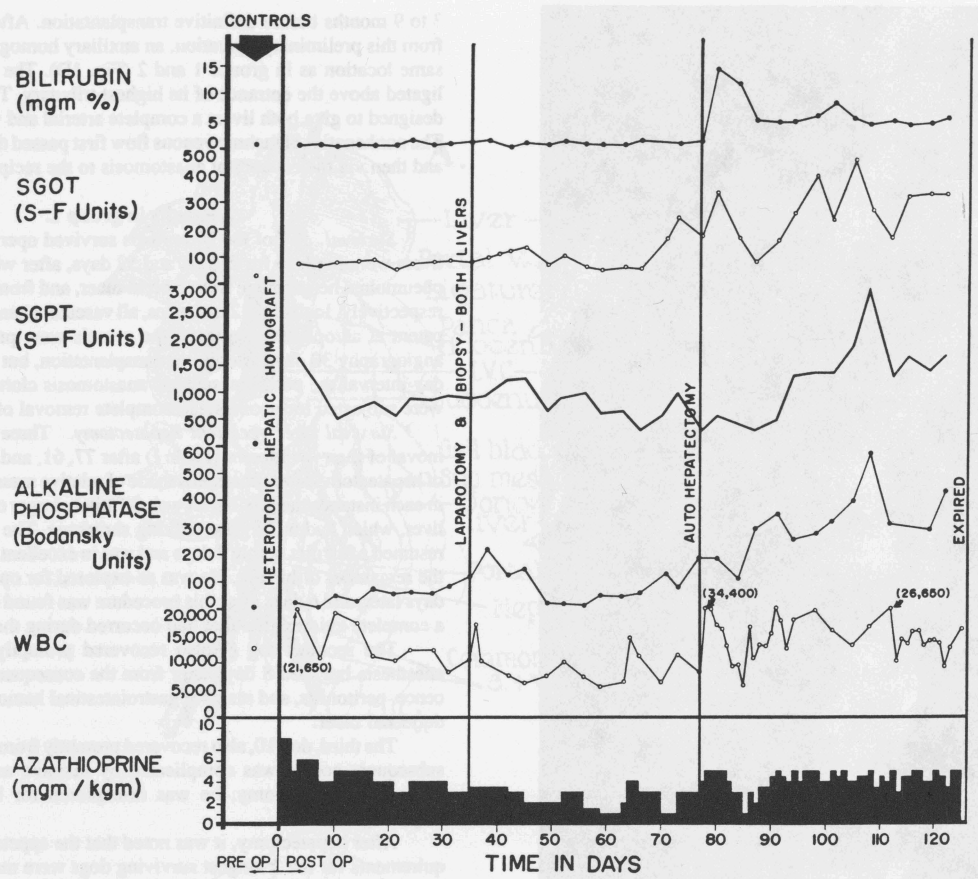

Fig. 3.— Clinical course of dog 5 of group 1 series. Note the abrupt bilirubinemia which followed removal of the dog's own liver, autohepatectomy. After autologous hepatectomy, the dog lived for 49 days with sole dependence upon the homograft, ultimately dying as the result of a wound dehiscence and evisceration which followed repeat biopsy.

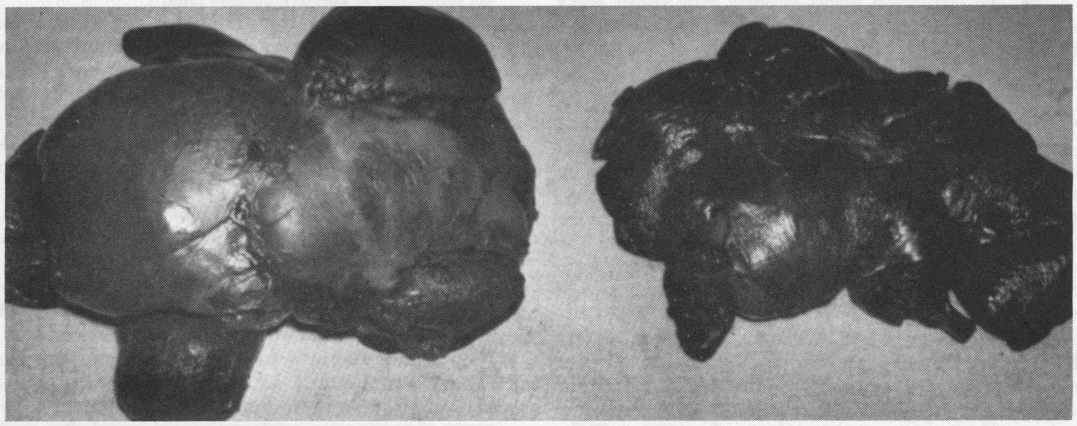

Fig. 4.— Auxiliary liver homograft, left, compared to the animal's own liver, right, in dog 8 of group 1 series. The homograft was obtained at autopsy, and the animal's own liver was removed 8 days earlier. The weight of the homograft was more than twice that of the autologous hepatic tissue.

TABLE I.—RESULTS IN GROUP 1

Dog No.	*Weight of donor kgm.*	*Weight of recipient, kgm.*	*Total survival, days*	*Removal host liver*	*Survival after hepatectomy, days*	*Weight of homograft at autopsy, gm.*	*Weight of host liver at autopsy or hepatectomy, gm.*
2	14.1	15.2	28	No	Not done	325	322
4	14.5	19.5	25	No	Not done	380	306
5	15.4	18.2	126	Yes	49	656	225
8	18.6	20.5	69	Yes	8	478	211
9	18.2	18.6	52	No	Not done	440	300
10	20.0	22.9	101	Yes	28	492	310

Gross pathologic observations. The weights of the animal's own liver and those of the homograft are listed in Table I. In each instance, the homograft weighed more than the host liver, despite the fact that the donor animals were all smaller than the recipients. In 3 animals, dogs 5, 8, and 10, the comparative weights of the autologous liver and the homograft were not obtained simultaneously, the interval from hepatectomy to death being 8 to 49 days. In the other 3, both weights were obtained at autopsy.

The animal's own liver in each instance was grossly normal except for it's small size (Fig. 4). The homografts in 5 instances appeared to be relatively normal (Fig. 4) except for some increased firmness. In the one dog in which thrombosis of the portal system occurred following angiography, the homograft was nodular and appeared cirrhotic and partially necrotic at autopsy.

Microscopic findings in host liver. Samples of host liver were examined microscopically 25, 26, 28, 30, 35, 51, 52, 61, 63, 73, and 77 days following high ligation of the portal vein and hepatic homotransplantation. Five of the 11 specimens were obtained either at open biopsy or at the time of removal of the host liver, while the others were collected at autopsy.

In the earlier samples there was necrosis of a variable number of the cells in the central zones of the hepatic lobules (Fig. 5A). In a few of the specimens this was associated with fat droplets in the cytoplasm of the liver cells in the other zones. The small branches of the portal vein were distended with blood. Large amounts of hemosiderin were present in the Kupffer cells and within macrophages which lay in the portal tracts.

In the samples collected after the thirty-fifth postoperative day there was centrizonal atrophy of the hepatic cells with collapse of the reticulin network immediately adjacent to the central vein (Fig. 5B). The bile ducts and hepatic arteries were normal. There were no infiltrating lymphoid cells. Iron pigment was still present.

Microscopic findings in the auxiliary homograft. Samples of the homografts were examined microscopically 25 to 126 days following transplantation, either at open biopsy, at the time of removal of the host's own liver, or at autopsy. Only autopsy tissues were available from dogs 2 and 4, 28 and 25 days respectively after transplantation. Dog 9 had a biopsy 30 days after homotransplantation; the portal venous anastomosis subsequently thrombosed, and the dog died 52 days after the original operation. Serial specimens were available for the other 3 dogs before and after removal of the host's own liver and at subsequent autopsy. These were obtained from dog 5 after 35, 63, 120, and 126 days. In dog 8 the tissues were taken after 26, 61, and 69 days. In dog 10 the specimens were obtained after 51, 73, 100, and 101 days. In dog 9 in which thrombosis of the portal system occurred after angiography, there was widespread centrizonal necrosis accompanied in several areas by necrosis of whole lobules and collapse of the reticulin framework.

In 4 of the other 5 liver transplants, there was cellular infiltration (Fig. 6A). In dog 2, which lived for 28 days, this affected only the small portal tracts, but in the others the adventitia of the central hepatic veins was also involved. The infiltrate consisted of small and medium-sized lympho-

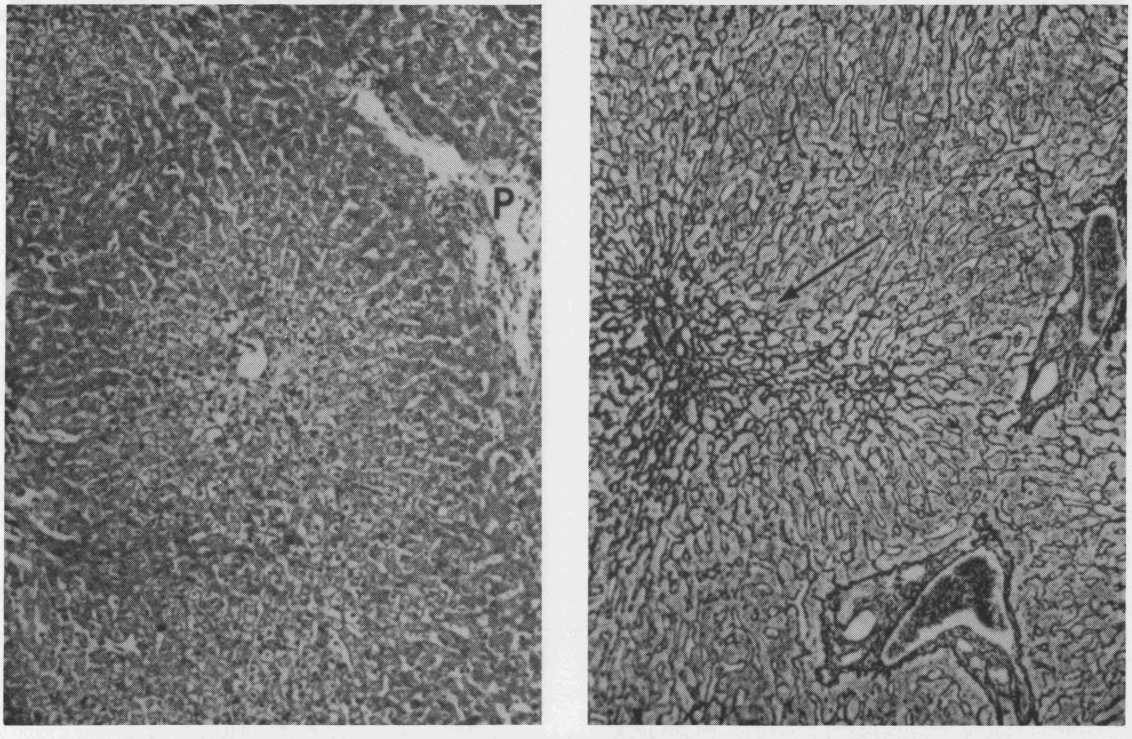

Fig. 5.— Biopsies of their own livers from 2 dogs in group 1: A, left, 35 days after operation (dog 5). There is centrizonal necrosis of the liver cells; P, portal tract. Hematoxylin and eosin, X65. B, right, 51 days after operation (dog 10). The centrilobular reticulin has collapsed (arrow). Reticulin, X90.

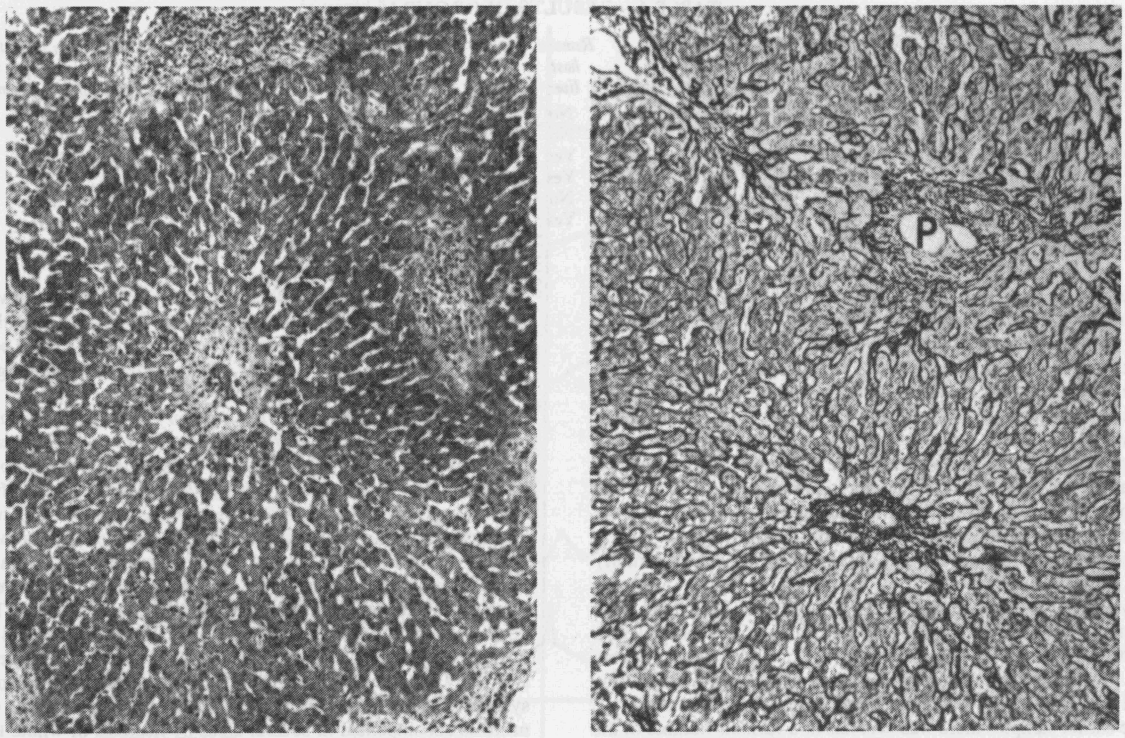

Fig. 6.— Biopsies of hepatic homografts from 2 dogs of group 1: A, left, 35 days after transplantation (dog 5). The portal tracts contain many infltrating cells. The wall of the central hepatic vein is thickened and lightly infiltrated with cells. Hematoxylin and eosin, X65. B, right, 61 days after transplantation (dog 8). There is a dense accumulation of reticulin and collagen fibers around and within the wall of the central hepatic vein; *P*, portal tract. Reticulin, X65.

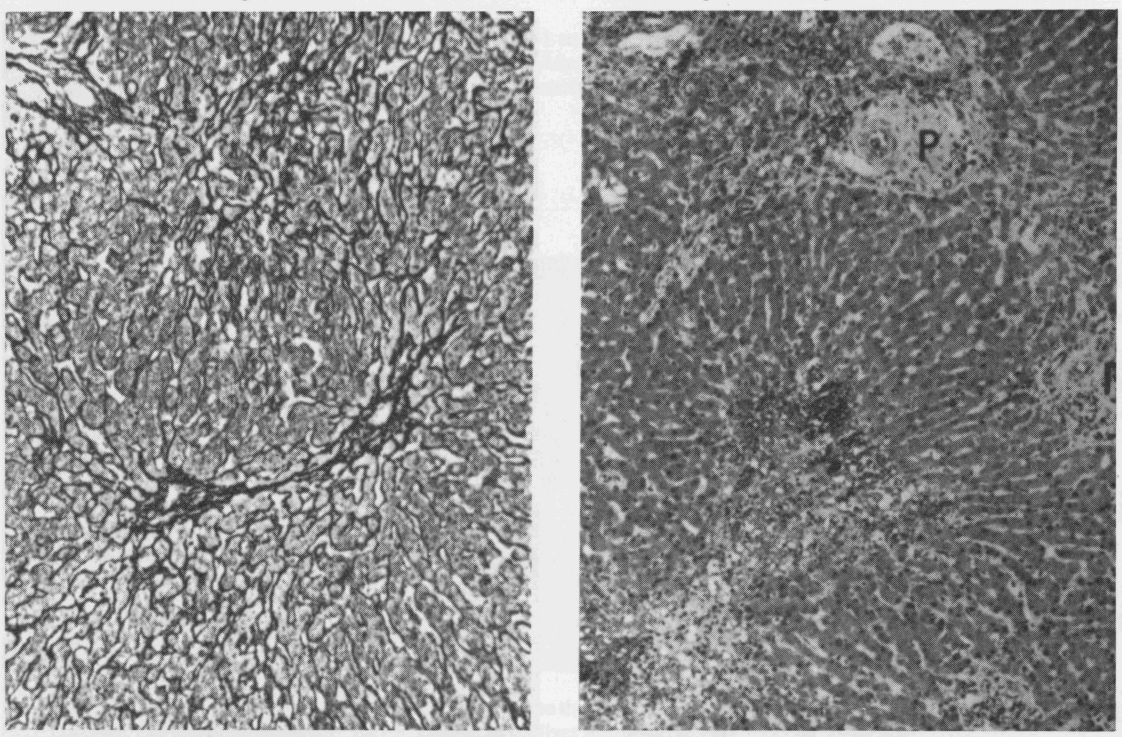

Fig. 7.— Two canine hepatic homografts: A, left, 120 days after transplantation (dog 5 group 1). A band of reticulin fibers links 2 central veins. Reticulin, X65. B, right, 52 days after transplantation (dog 18 group 2). Centrizonal liver cell necrosis and hemorrhage is combined with cellular infiltration of the portal tracts (P). Compare with Figure 6A. Hematoxylin and eosin, X65.

TABLE II.—RESULTS IN GROUP 2

Dog No.	*Weight of donor, kgm.*	*Weight of recipient, kgm.*	*Total survival, days*	*Weight of homograft at autopsy or sacrifice, gm.*	*Weight of host liver at autopsy or sacrifice, gm.*
14	14.7	19.0	46	285	390
15	15.6	18.6	41	400	475
16	15.0	24.1	57*	118	485
18	10.9	15 9	52*	150	358
19	11.7	15.0	50*	158	265
20	15.4	19	58*	346	262

*Sacrificed

cytes, plasma cells, and larger cells with scanty pyroninophilic cytoplasm, large nuclei, and prominent nucleoli. The only unaffected liver was that of dog 4 which lived for 25 days. There was a tendency as time went by for the cellular infiltration to diminish in intensity and for plasma cells to predominate. For example, in dog 5 in which the infiltrate was very heavy at 35 days, there was a slight diminution by 63 days, but by 120 days invading cells were far less frequent and were then mostly plasma cells. A similar striking decrease was seen in dog 8 in which the biopsy taken at 61 days showed much less cellular infiltration than was present at 26 days.

When cellular infiltration involved the central hepatic veins, there was swelling of the endothelial cells and, in 2 of the dogs, fibrinoid necrosis of the venous wall was present. In biopsies, these veins were the site of fibrous intimal thickening and accumulation of reticulin and collagen fibers around the adventitia of the affected vessels (Fig. 6B). Fibrous occlusion of some central veins was found in dogs 5 and 8.

A few foci of acute fibrinoid necrosis were seen in the walls of small hepatic arteries in dog 2; in the other dogs these vessels were normal. The sinusoids were distorted in the central zones of the liver from dog 2, and in this same animal the Kupffer cells were loaded with hemosiderin. In the other 4 homografts the sinusoids were normal and the Kupffer cells contained very little iron pigment. The portal veins and lymphatics were normal in all the homografts.

In the 1 animal, dog 2, which died from massive gastrointestinal hemorrhage there was necrosis of liver cells in the central zones of the lobules. In the other homotransplants, the liver cells appeared normal.

In the biopsies from dogs 5 and 10 at 120 and 100 days, respectively, fibrous and reticulin bands linked adjacent areas of centrilobular perivenous fibrosis (Fig. 7A). There was also slight periportal fibrosis. The lobular architecture was normal in the other three hepatic homotransplants.

Small bile ducts became increasingly difficult to identify in dogs 5 and 10 after 63 and 73 days, respectively, and this fact was associated with the appearance of inspissated bile in the centrilobular canaliculi. In the earlier biopsies from these dogs and in the other homotransplants, bile ducts and canaliculi were normal.

Results in group 2.

Survival. Six of the 10 dogs survived the immediate postoperative period. Two of the 6 remaining dogs died spontaneously. The first lived for 41 days and died 4 days after biopsy and direct inspection of the arterial anastomosis. At operation, a needle was inserted into the hepatic artery for pressure determination. At postmortem examination the vessel was found to have a fresh thrombosis. Pneumonia was also present. The other animal died at 46 days of bilateral pneumonia.

The other 4 animals were sacrificed from 50 to 58 days after homotransplantation, usually following biopsy and angiography.

Angiographic studies. Four of the 6 dogs had angiograms. In these 4, the vascular anastomoses were patent and blood flow above the mesenteric ligature was demonstrated to the animal's own liver (Fig 8A). Flow below the ligature passed to the homograft (Fig. 8B).

Biochemical studies. These were essentially the same as in group 1 (Fig. 9). In none of the dogs did jaundice develop. No second stage host hepatectomies were carried out.

Gross pathologic observations. In dog 14 the portal vein was found at autopsy to be thrombosed. In this experiment, the dog's own liver weighed 390 grams, and the homograft weighed 285 grams.

In the other 5 animals, in which the portal branches were patent, there was variation in the sizes of the homograft and the animal's own liver (Table II). In dog 20, the homograft was distinctly larger, being 346 grams compared to 262 grams for the host liver. In the other 4, the host liver was larger, but in one of these, dog 15, the difference was slight, the homograft weighing 400 grams compared to 475 grams for the autologous organ. In the other 3, the disparity was greater, the homografts weighing 158, 150, and 118 grams compared to autologous liver weights of 265, 358, and 485 grams, respectively (Table II).

Microscopic studies in host liver. Samples of host liver were examined microscopically 37, 39, 41, 46, 49, 50, 52, 55, 57, and 58 days following ligation of the superior mesenteric vein and hepatic homotransplantation. Six of the 12 specimens were obtained at open biopsy, while the others were collected at autopsy.

Four of the livers were normal. The liver from dog 18, which had suffered a serious blood transfusion reaction, showed congestion of the sinusoids in the centers of the lobules and necrosis of the liver cells adjacent to the central veins which were congested. Inspissated bile was present in some of the centrilobular canaliculi. In the greatly atrophied liver from dog 20 there were the same changes of centrizonal atrophy of the hepatic cells with collapse of the reticulin network adjacent to the central vein as were seen in the host livers of group 1.

Microscopic findings in the auxiliary homografts. Samples of the homografted livers were examined at the same time after transplantation as were specimens from the dogs own livers. In 2 of the livers large vessels were thrombosed. The biopsy at 39 days from dog 14 showed inspissated bile in the centrilobular canaliculi and large deposits of hemosiderin in the Kupffer cells, but no other abnormalities. Seven days later, after portal vein thrombosis, there was extensive centrizonal hemorrhage and necrosis of liver cells. In dog 15, hepatic artery thrombosis produced widespread hemorrhagic necrosis of the liver.

Of the other 4 homotransplants, the 3 livers which underwent atrophy all showed centrizonal necrosis of liver cells (Fig. 7B). In dog 16 this was present in the first biopsy taken at 37 days and at the time the liver cells in the middle and peripheral zones contained many fat deposits in their cytoplasm. By 55 days the necrotic process had spread so that in many areas only the periportal liver cells remained. In dogs 18 and 19 the process was not so severe. The liver from dog 20 showed no centrilobular necrosis of liver cells.

Infiltration with lymphoid cells was present around the smaller branches of the portal vein and around the central veins in these 4 hepatic homotransplants. In dog 16 the number of infiltrating cells was at first large, but in the biopsy taken at 57 days this infiltration was greatly diminished. Slightly fewer cells were present in dogs 18 and 19, and very few were present in dog 20.

As in group 1, cellular infiltration around the central veins was accompanied by swelling of endothelial cells and in 2 instances by focal fibrinoid necrosis of the vein wall. Perivenous fibrosis and occlusion of the lumina of the central veins subsequently occurred in dogs 16 and 19.

Portal veins were normal. There were foci of acute fibrinoid necrosis in the wall of a small hepatic artery (Fig. 10) and eccentric intimal fibrous thickening in another in 1 homografted liver at 50 days, dog 19. The sinusoids were interrupted in the liver lobules of the 3 animals showing centrilobular necrosis. Hemosiderin was present in large amounts in both Kupffer cells and in macrophages lying in the portal tracts in the livers from dogs 16 and 18. The lymphatics were normal in all the homografts.

There was collapse of the reticulin network of the lobules adjacent to the central vein in the 3 dogs which showed centrilobular loss of liver cells. In the specimens from dogs 18 and 19 at 52 and 50 days respectively, fibrous and reticulin bands linked adjacent areas of fibrosis around central veins. In dog 16 there was complete collapse of the reticulin framework wherever necrosis of all the liver cells in a lobule had occurred. The lobular architecture was undisturbed in dog 20.

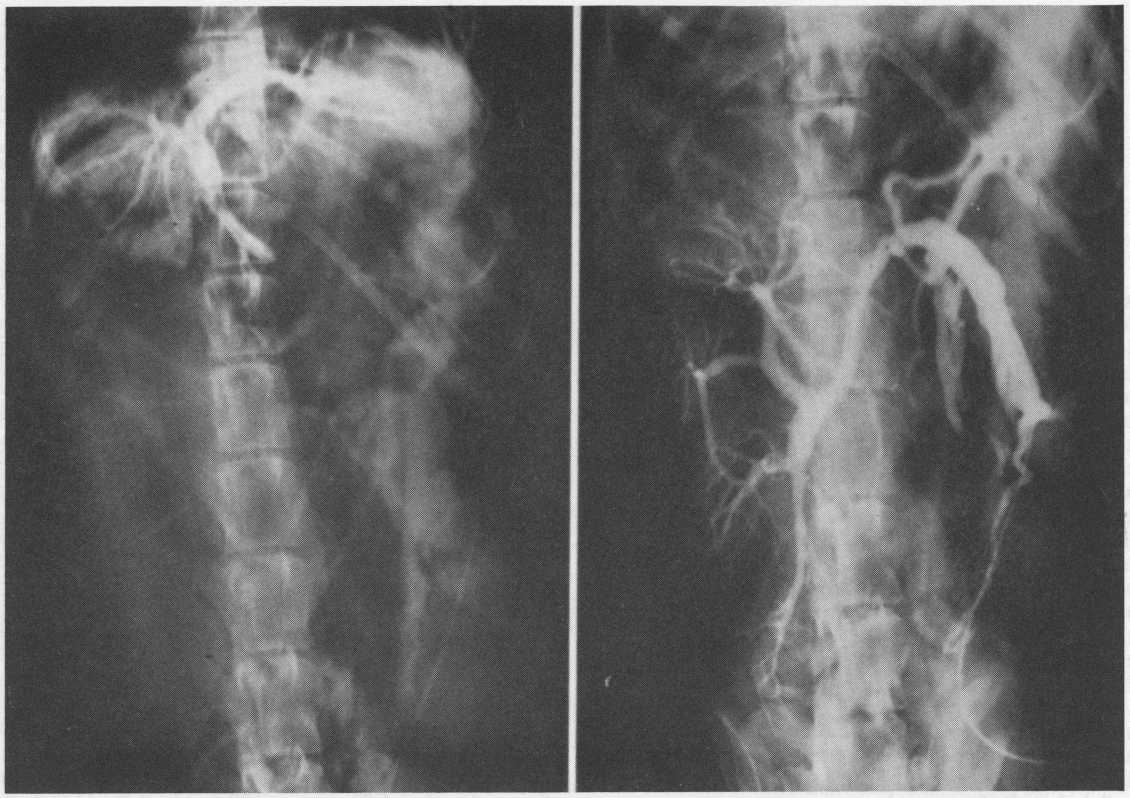

Fig. 8.— Operative angiograms on dog 20 of group 2. A, left, Contrast medium injected into the splenic vein passes through the animal's own liver. B, right, Contrast material injected through a distal mesenteric vein tributary passes through the homografted liver. Note the relatively large size of the homograft compared to the animal's own liver. This impression was confirmed at autopsy at which the homograft weighed 346 grams and the autologous liver, 262 grams.

In the homograft from dog 16, many of the small bile ducts were severely damaged, and this was accompanied by accumulations of bile pigment in the surviving liver cells. Proliferation of the smaller bile ducts was present in the homgraft from dog 19. In the other 2 animals, the bile ducts and canaliculi appeared normal.

Results in group 3.

Survival. A completely satisfactory technical result was not obtained in any of the 6 experiments. Three dogs died immediately after operation. Late thrombosis of the homograft vena cava was observed in 2 of the remaining dogs, and late thrombosis of the portal vein in the third animal.

Discussion.

Since the description of auxiliary hepatic homotransplantation by Welch and Goodrich and their associates, a number of studies of this preparation have been published. The assumption has been generally made that the changes observed in the grafted tissue were solely due to immunologic rejection. Virtually all of this earlier work was carried out in canine recipients which were treated with immunosuppressive agents, including Welch's original studies and those subsequently reported by Sicular and Mehrez and their associates. Under these circumstances, the influence of physiologic factors upon the outcome was difficult to delineate because the full vigor of homograft rejection destroyed the transplanted tissue within a few days.

The only reports in the literature of auxiliary homotransplantation to recipients which were receiving immunosuppressive drug therapy were published from this laboratory.[12,13] In dogs, it was found that the behavior of such secondarily placed homografts was significantly different from that of orthotopic homografts in similarly treated animals in that the auxiliary homograft markedly diminished in size within a few weeks after operation. The present study provides strong evidence that this difference in behavior in the two types of homografts is not solely of immunologic etiology. Rather, the auxiliary homograft shrinkage appears to be due to deprivation of nonhepatic splanchnic flow in the presence of the recipient animal's own liver. When, as in the present study, the situation is reversed so that the nonhepatic splanchnic flow is directed initially through the homograft, a similar shrinkage is observed in the dog's own liver. These experiments have made clear at least one physiologic requirement which must be observed in revascularization of the auxiliary liver homograft. Because of the apparent competition for nutritional substrate which occurs in the presence of 2 livers, the homografted organ must receive its portal supply directly from the alimentary venous return.

The histologic changes in the recipient's own liver are of great interest in the dogs of group 1. During the entire course of these animals, serial biochemical changes were described, but it was, of course, impossible to be sure if these resulted from homograft injury or from damage to the autologous liver. The small size of the host livers in these animals, in which the splanchnic flow was diverted completely through the homograft, was apparently due to centrizonal necrosis with collapse and condensation of reticulin in the center of the lobule. These changes are similar to those described by Mann and his associates more than 30 years ago, after the performance of Eck fistula in dogs, an experiment in which immunologic factors were absent. In the host livers of the present study, there was nothing to suggest that they had been damaged by a graft versus host reaction; lymphoid cell infiltration and arterial lesions were not seen. Also, although it is known that azathioprine can cause hepatic damage, the normal state of the majority of the dogs' own livers in group 2 makes it improbable that chronic drug toxicity was an important factor. It is interesting that the only host liver in group 2 which showed more severe centrilobular lesions and general atrophy came from dog 20 in which the homograft had retained its full size.

The same substrate starvation, which appeared to have caused this

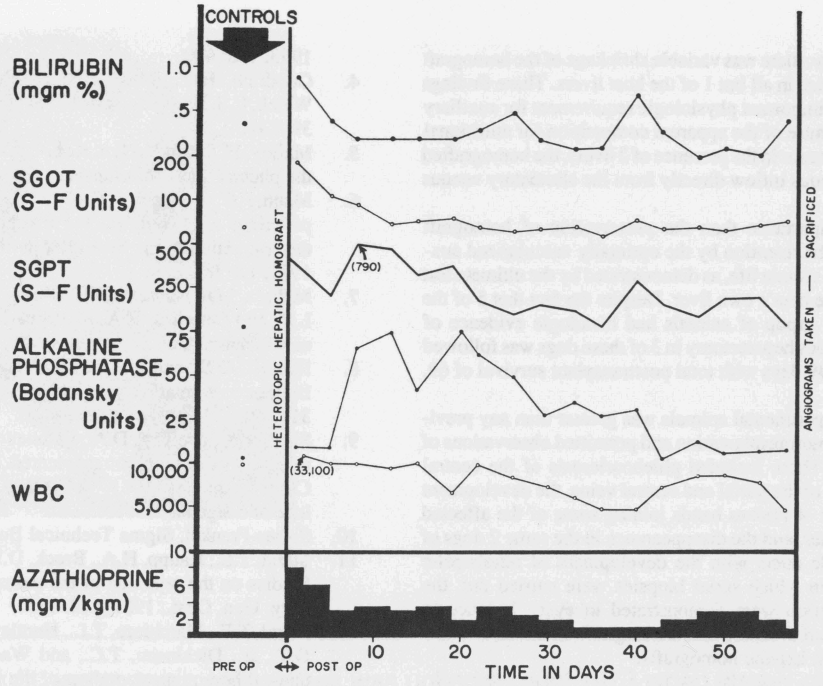

Fig. 9.— Clinical course of dog 20 of group 2. The animal is the same one as shown in Figure 8.

damage to the host liver, seemed to have an even more adverse effect upon the homograft, particularly when judged by the gross characteristic[sic] and weight of the competing livers. In the animals of group 2, in which the 2 livers shared the nonhepatic splanchnic flow, 5 of 6 homografts were smaller than the respective host livers and in the 3 most severely atrophied transplants, in this series, the same centrizonal necrosis was very prominent. Because the homograft is subject to the injury of rejection, the resultant histological alterations cannot be so clearly assigned a metabolic etiology, however, especially since centrizonal necrosis has also been described as characteristic in the rejecting orthotopic hepatic homograft, either with or without use of immunosuppresive drugs.[11,13]

Long survival of some of the animals in the present study permitted analysis of the pathologic findings at a much later time than has previously been possible and allowed a serial view of the histological changes. In the first 2 experimental groups, the majority of the homotransplanted livers showed evidence of a cellular homograft reaction with lymphoid cell infiltration around the small branches of the portal vein and around the central hepatic veins. In all of these homotransplants that were sequentially biopsied, the infiltration tended to decrease with time, a process which was not appreciably hastened following removal of the host's own liver.

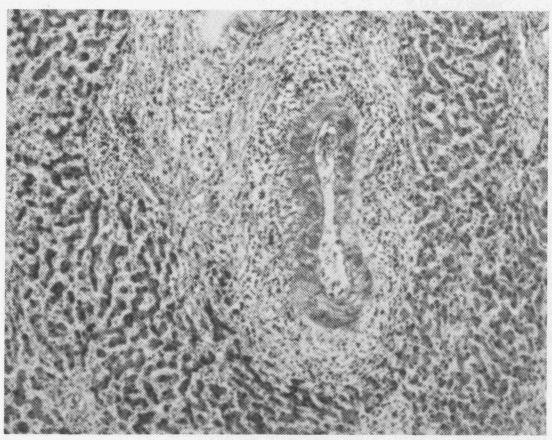

Fig. 10.— Homografted liver 50 days after transplantation (dog 19 group 2). In the portal tract a branch of the hepatic artery shows fibrinoid necrosis of its wall. The surrounding connective tissue is infiltrated with cells. At the right of the photomicrograph there is centrizonal necrosis of liver cells. Hematoxylin and eosin, X50.

Late in the course, centrilobular fibrosis and phlebosclerosis and, to a much lesser extent, periportal fibrosis were seen. In the longest survivors, these changes had progressed to the development of fibrous bands which linked some of the affected central zones. In many ways, the evolution of these structural alterations resembled that of veno-occlusive disease such as that described by Bras and his associates. Another late finding was the loss of small bile ducts with consequent accumulation of inspissated bile in the centrilobular canaliculi. Fibrinoid necrosis of branches of the hepatic artery was rare. It is probable that these late changes occurred as the result of the action of circulating and cell-bound antibodies produced by the host, but at present it is still difficult to distinguish immunologically mediated lesions from those which result from mechanical or other factors. The central phlebosclerosis and surrounding fibrosis could, for example, have been the result of a mild chronic degree of early postoperative outflow block. As more long term survivors from canine hepatic homotransplantation are studied, these questions concerning the late pathologic findings in the homografts should be resolved.

Summary.

Auxiliary hepatic homotransplants were performed in dogs, the second liver being placed in the pelvis of the recipient. In each instance, the hepatic artery was revascularized from the external iliac artery. In 6 dogs, the portal vein of the homograft was anastomosed to the superior mesenteric vein and the proximal portal vein then ligated; the splanchnic blood flow was directed in a retrograde fashion through the homograft, and the dog's own liver was left supplied solely by the hepatic artery. In another 6 dogs, a similar operation was performed and venous revascularization carried out so that the non-hepatic splanchnic flow of the recipient was shared by both the host liver and the homograft.

In those animals in which the nonhepatic splanchnic flow was diverted through the auxiliary liver (group 1), the homograft retained its full size and there was marked shrinkage of the host's own liver. Histologically, the loss of size observed in the host liver appeared to be due to centrizonal necrosis with collapse of the reticulin which was most pronounced around the central vein.

In those animals in which the host liver and the homograft shared the

nonhepatic splanchnic flow, there was variable shrinkage of the homograft with retention of normal size in all but 1 of the host livers. These findings appear to have defined an important physiologic requirement for auxiliary homotransplantation. Because of the apparent competition for nutritional substrate, which seems to occur in the presence of 2 livers, the homografted organ must receive its venous inflow directly from the alimentary venous return.

Of even greater importance than the preservation of homograft morphologic integrity is the retention by the optimally vascularized auxiliary liver of the ability to sustain life, as demonstrated by the ultimate test imposed by removal of the host's own liver. Despite the fact that 5 of the 6 homografts in the first group of animals had histologic evidence of cellular rejection, autologous hepatectomy in 3 of these dogs was followed by survival of 8, 28, and 49 days with total posttransplant survival of 69, 101, and 126 days.

Survival in these experimental animals was greater than any previously reported hepatic homotransplantation and permitted observations of late pathologic changes. These included phlebosclerosis of the central veins, perivenous fibrosis of the portal and central veins, the development in the 2 longest survivors of fibrous bands joining some of the affected central zones one to another, and the disappearance in the same 2 dogs of the small intrahepatic bile ducts with the development of intrahepatic cholestasis. In the dogs in which serial biopsies were carried out, the findings of cellular rejection were demonstrated in every instance to diminish with the passage of time, the first histologic demonstration of the reversibility of rejection in hepatic homografts.

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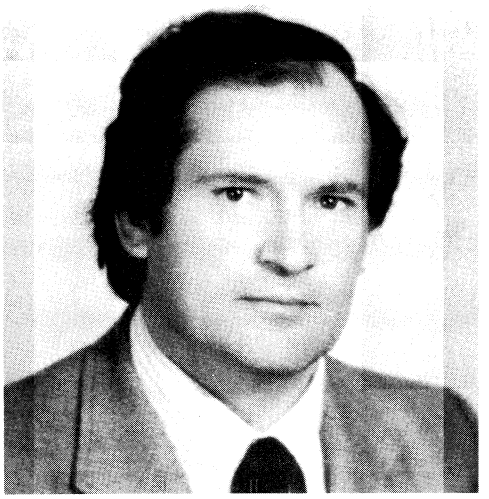

Antonio Francavilla

The nature of hepatotrophic substances was unknown until 1973 when strong circumstantial evidence that they were hormones, especially insulin, was provided from so-called splanchnic division experiments. The principle of the experiments was to divide the liver into fragments with differing splanchnic venous input from various intraabdominal organs. The liver fragment exposed to the venous effluent from the pancreas always was healthier than the other fragments suggesting that pancreatic hormones (especially insulin) were the critical hepatotrophic substances. The biochemical studies were provided by the brilliant Italian physician, Professor J. Antonio Francavilla, Chairman of the Department of Hepatology, University of Bari, Italy. The way in which Dr. Francavilla's biochemical studies supported and extended the histopathologic observations of Dr. Porter was a classic demonstration of the power of interdisciplinary research. Dr. Francavilla's work in this field has continued to the present time and currently is concerned with the biochemical events and control of hepatic regeneration.

The origin, hormonal nature, and action of hepatotrophic substances in portal venous blood

Surgery, Gynecology & Obstetrics, 137: 179-99, 1973

T. E. Starzl, A. Francavilla, C. G. Halgrimson, F. R. Francavilla, K. A. Porter, T. H. Brown and C. W. Putnam

Long ago, Rous and Larimore(39) were intrigued with the possibility that portal venous blood contained hepatotrophic factors and that the extrahepatic diversion of these factors by portacaval shunt was responsible for the poor health of dogs with Eck fistula. However, the observations of Mann(27) did not support the hepatotrophic hypothesis, and the work in 1953 of Child and his associates(11) using portacaval transposition was generally interpreted as crucial evidence against it. By replacing the diverted splanchnic venous blood with an inflow to the portal vein from the inferior vena cava, Child avoided most of the adverse effects of Eck fistula. The concept became rooted from these studies and those of Fisher and his associates(15-17) and a number of subsequent authors that the quality of portal venous inflow was not a prime determinant of good hepatic structure, function, or capacity for regeneration. Instead, it became accepted that the quantity of total hepatic blood flow was the main consideration. In spite of the demonstration that canine livers submitted to transposition underwent major deglycogenation and were, thus, not actually normal(43,52), the flow oriented view held sway until it was definitively challenged by investigations that had their origin in studies of experimental liver transplantation as has been thoroughly reviewed in a recent monograph(50).

First, it was noted that auxiliary hepatic homografts underwent remarkable atrophy if these extra livers were revascularized in an ectopic location with a double systemic blood supply analgous to that with Child preparation(51). One possible explanation that was advanced was that the organ which was perfused first by splanchnic venous blood extracted a disproportionate share of unspecified substances and that the other organ atrophied because of its disadvantaged competitive situation. The hypothesis was supported by Marchioro and his associates(29) who showed that the transplant atrophy could be prevented by diverting the nonhepatic splanchnic venous blood away from the host liver and through the graft. By so doing, the atrophy now afflicted the native organ. Confirmatory observations were reported by Thomford(56), Halgrimson(20), and Tretbar(57) and their associates. Thomford(56) showed that the atrophy in Welch auxiliary homografts could be prevented in recipients which had undergone immunosuppression if the host livers were removed within a few days after transplantation, and Tretbar(57) and Halgrimson(20) and their colleagues demonstrated that the shrinkage could be reduced by the diversion of portal blood away from the host liver even though it was not directly rechanneled through the transplant. Observations by Sigel and his associates(47,49) with hepatic autografts implanted to intestinal pedicles or directly revascularized in the neck could be interpreted in the same general way.

The transplant preparations which made apparent the foregoing physiologic effects had two serious flaws which prevented definitive conclusions about the pathogenesis of the atrophy. First, the total flows delivered to the two coexisting livers were often different. Second, there was by definition an additional inherent inequality of the two organs since the homograft was usually under immunologic attack despite host immunosuppression whereas the animal's own liver was not. Consequently, other experiments were undertaken which were designed to circumvent one or both deficiencies.

One preparation not involving transplantation was used by Marchioro and his associates(28,30) and termed a split transposition. Splanchnic venous blood was provided for one portal vein branch of the liver whereas the other portal branch was supplied with blood from the inferior vena cava. Later, Price(36), Lee(25), and Chandler(9) and their associates performed analogous experiments, with either canine partial hepatic autografts or isografts of inbred rat livers. All these experiments showed hypertrophy in the hepatic tissue which was perfused with splanchnic blood and atrophy of the other hepatic fragments. In addition to hypertrophy, Marchioro and his associates(28) showed that the advantaged hepatic portion had binucleate and trinucleate hepatic cells, mitosis, and proliferating bile ducts, all indications of hyperplasia. With quantitative studies of deoxyribonucleic acid synthesis, Lee(25), Chandler(9) and Fisher(18) and their associates proved that the hepatotrophic effects of splanchnic blood upon the liver include hyperplasia as well as hypertrophy. It has become increasingly accepted that the portal hepatotrophic factors are probably not just artifacts of transplantation and other experimental maneuvers but are prime determinants of the initiation and control of the liver hypertrophy and hyperplasia in many circumstances.

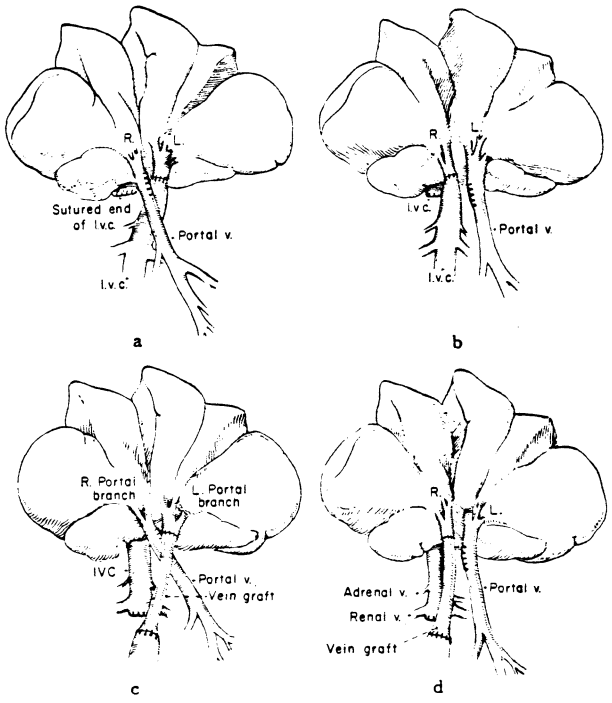

Fig. 1.— Partial portacaval transposition. a and b, Entire vena caval flow is directed into either the left or the right portal venous branch. c and d, This differs from a and b in that the vena caval flow excludes the adrenal and renal blood. A venous graft is always required to bridge the gap.

This article was undertaken to report anatomic and biochemical studies of the source and mechanism of the hepatotrophic factors in the splanchnic venous blood. The results have indicated that these originate from the pancreas and are hormonal in nature.

Methods

Portal Diversion Procedures

One hundred and one mongrel dogs, weighing 12.4 to 24.1 kilograms, were used. Six normal dogs were sacrificed to obtain tissues for control studies and liver lobe weights, and the remaining 95 dogs had one of the following operative procedures.

Group 1, Partial portacaval transposition. a, Split transposition. - In 15 dogs, the left (Fig. 1a) and in 18 dogs the right (Fig. 1b) portal vein was detached from the main portal trunk and revascularized by an end-to-end anastomosis to the supra-adrenal inferior vena cava by themethod of Marchioro and his associates(28). The procedure divides the liver into two compartments which are dissimilar in that one receives portal blood from the total splanchnic venous bed and the other obtains its portal supply from systemic sources, including the effluent from the kidneys, adrenals, and hindquarters.

b, Split transposition minus adrenal and renal inflow.-The procedure was identical to that just described except that the systemic venous blood was derived from the infrarenal inferior vena cava thereby excluding the renal and adrenal effluent. This blood was transmitted to the appropriate branch of the portal vein by way of an internal juglar vein graft. In six dogs, the systemic venous input was to the left portal branch (Fig. 1c) and in two dogs, to the right portal vein (Fig. 1d).

Group 2, splanchnic flow division. The two portal branches were isolated. One was left undisturbed; the other was detached and anastomosed by means of an iliac vein graft to the common mesenteric vein below the level of the splenic and pancreatic venous input. Proximally, the mesenteric trunk was ligated just below the splenic vein (Fig. 2). Thus, one side of the liver received portal blood of an intestinal source, and the other side received venous blood returning from the pancreatic, splenic, and gastroduodenal beds. Twenty experiments each were performed to the right and left sides.

In the dog, the pancreas has two distinct lobes. Early in the series, it was discovered that the tail of the inferior pancreatic lobe almost invariably drains into the mesenteric venous circulation (Fig. 2, insets). Thereafter, this portion of the pancreas was always resected at the time of the splanchnic division procedure. Three dogs operated upon before this observation was made had delayed partial pancreatectomy at the time of the first biopsy one month after the original operation.

Group 3, total portacaval transpositions. a, Standard transposition.- Nine dogs underwent portacaval transposition by means of Child's method(11) and as shown in Figure 3a. An open liver biopsy was perfomed before the transposition.

b, Total transposition minus adrenal and renal inflow.-Five dogs had a modified portacavaltransposition (Fig. 3b) with a revascularization of the portal vein by means of a venous graft from the infrarenal inferior vena cava.

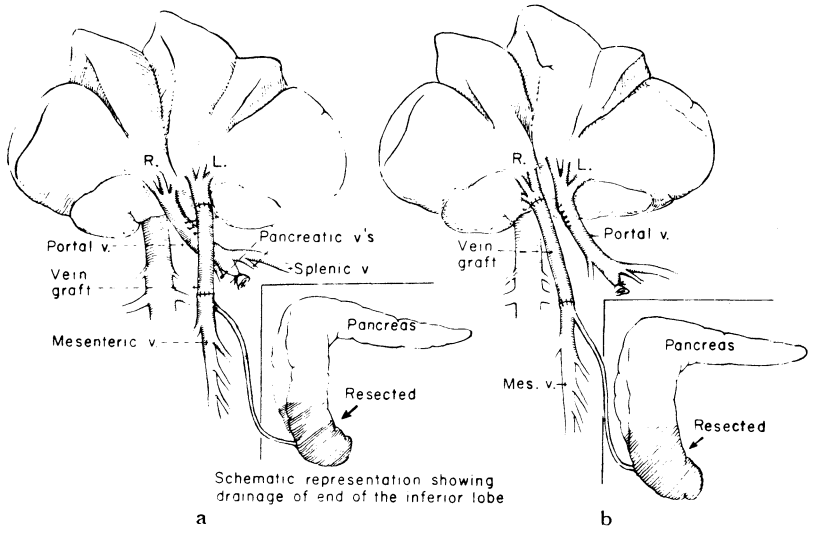

Fig. 2.— Technique of division of splanchnic venous flow into a pancreatico-gastroduodenal-splenic compartment and an intestinal compartment. Blood from these respective sources is directed into the right or left lobes. The tail of the inferior lobe of the pancreas was resected since it drains separately into the mesenteric vein.

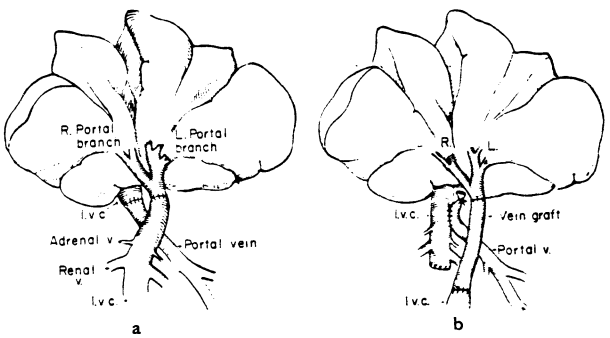

Fig. 3.— Techniques of transposition for the dogs in group 3. a, Standard portacaval transposition of Child. b, Modified transposition which eliminates adrenal and renal venous blood from the portal blood samples. A venous graft is always required.

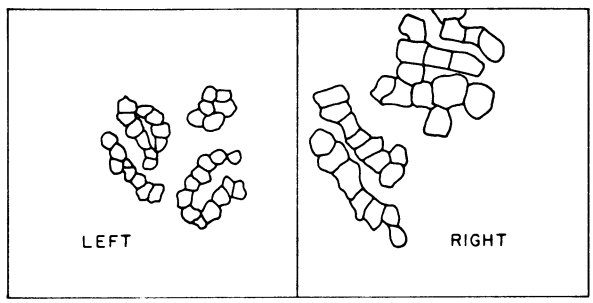

Fig. 4.— Hepatocyte shadows traced during histopathologic examination. These were later cut out on standard paper and weighed as an index of hepatocyte size. The specimens depicted were from the experimental group 2 (see Fig. 2a). The right lobes with the large hepatic cells received venous blood from the pancreas, stomach, duodenum, and spleen. The relativly shrunken left lobes with the small hepatocytes received intestinal blood.

Postoperative Studies

The dogs were maintained on a standard kennel diet. Bilirubin, alkaline phosphatase, and serum glutamic oxalacetic transaminase values were checked twice a week. These values were never abnormal.

Vessel patency. Prior to liver biopsy, the patency of the anastomoses was determined either by angiography or at exploratory laparotomy. At angiography of the dogs in groups 1 and 2, an effort was made to see if dye injected in one venous pool spilled into the other bed. Complete gross separation was always demonstrated.

Liver biopsies. In all dogs found to have patency of the two portal branches, biopsy specimens were taken from both sides. To minimize any effects due to the anesthetic agents, the specimens were removed under normothermia as soon as possible after induction of anesthesia with phencyclidine hydrochloride (Sernylan®), atropine, and pentobarbital sodium. The liver was first carefully examined for gross evidence of hypertrophy or atrophy. Blood flow to the area of the biopsy was not interfered with in any way until the specimen had been removed. In all experiments except those of group 3, both sides of the liver were biopsied and the specimens processed separately. Two grams of liver tissue were excised. One and one-half grams of tissue were snap frozen within five seconds and stored in liquid nitrogen at -158 degrees C. until the biomedical studies were performed. The rest of the biopsy specimen was used for pathological studies. A portion was fixed with formalin, and the remaining tissue was frozen in dry ice.

Autopsy procedures. Most of the dogs were sacrificed after the last biopsy. In the dogs in group 1 and 2, the liver was excised and extraneous tissue, including the gallbladder, trimmed from it. After weighing the entire liver, the two portal branches were carefully dissected out and a decision made as to the exact portal venous distribution to each of the lobes and sublobes of the liver. When the entire liver had been thus subdivided into the right and left components, it was cut along the axis and the right and left portions weighed separately. The normal weight ratio for the right lobes versus the left lobes had been shown by Child(11), Marchioro(28), and Pouyet(34) and their associates to be about 30:70. Of the six normal control dogs which were sacrificed, these same proportions were verified as will be noted later.

In dogs dying prior to biopsy, the same morphologic evaluation for atrophy and hypertrophy was performed, and specimens were taken for histologic studies, but no arrangements were made for biochemical determinations.

Criteria for hypertrophy. On histopathologic study, relative atrophy or hypertrophy of the different liver portions was usually evident, and differences of a lobule size, fat content, reticulin pattern, and glycogen content could be detected with the appropriate stains.

To obtain a quantitative estimation of the hepatocyte size, a tracing device was attached to the light microscope, and large numbers of hepatocytes in each experiment were drawn on a standard thickness paper. Forty representative traced hepatocytes were then cut out, and the pieces of paper they occupied were weighed (Fig. 4). The weight in grams was used to denote size units. We have shown this to be an accurate method for comparing cell sizes by confirmatory planimetry and by studies of unicellular organisms, the size of which could be directly determined.

Criteria for hyperplasia. The following hallmarks of hyperplasia were looked for: increased numbers of mitoses, the presence of binucleate and trinucleate hepatocytes, increased numbers of bile ductules, and increased thickness of the hepatic cell plates.

Biochemical Determinations

Hepatic glycogen. The method of Bloom and his associates(4) was used to separate the trichloroacetic acid soluble glycogen fraction from the insoluble one. Both fractions were quantitated with the anthrone method of Seifter and his colleagues(42), and the results were expressed in milligrams of glycogen per gram wet weight of liver.

Active and total phosphorylase. The active form of hepatic phosphorylase, Enzyme Commission Number 2.4.11., was measured by the method of Shimazu and Fukada(45), wherein additional activation during the assay of phosphorylase, Enzyme Commission Number 2.7.1.38., is prevented by inhibiting dephosphophosphorylase kinase and phosphorylase phosphatase, Enzyme Commisssion Number 3.1.3.17, with ethylenediaminetetra-acetate and sodium flouride, respectively. The assay for the phosphorylase determination contained 50 millimoles of glucose-1-phosphate and 1 per cent glycogen, to which a glycerol extract of liver was added. Incubation was for ten minutes at 37 degrees C., following which the inorganic phosphate released was measured by the method of Takahashi(55). The activity of the phosphorylase was expressed as millimicromoles of phosphate liberated in one minute by 1 milligram of protein of liver extract.

Total phosphorylase activity was assayed in the same way except that inactive phosphorylase was first rendered active with adenosine triphosphate according to the method described by Shimazu and Amakawa[44].

Hepatic cyclic 3', 5'-adenosine monophosphate (cyclic AMP). The method for extracting the liver sequentially with trichloroacetic acid and cold water-saturated diethyl ether has been described by Wastila and his associates(60). The extract was directly assayed for cyclic 3', 5' -adenosine monophosphate by the protein binding method of Gilman(19). The values obtained represent the means of four determinations of each sample and are expressed as picomoles per gram wet weight of liver.

Protein concentration. The protein of a weighed liver specimen was extracted with 10 per cent trichloracetic acid and digested with 3 per cent desoxycholic acid in sodium hydroxide. The protein concentration was then measured with the biuret method of Henry and his colleagues(21).

Protein synthesis. Twenty-four hours before biopsy, the dogs were given an intravenous injection of 60 millicuries of ^{14}C-leucine which had a specific activity of 28.1 millicuries per millimole. A 70 to 100 milligram portion of the biopsy specimen was processed by the method of Schneider and Hogeboom(41) as modified by Siekevitz(46), and the resulting protein

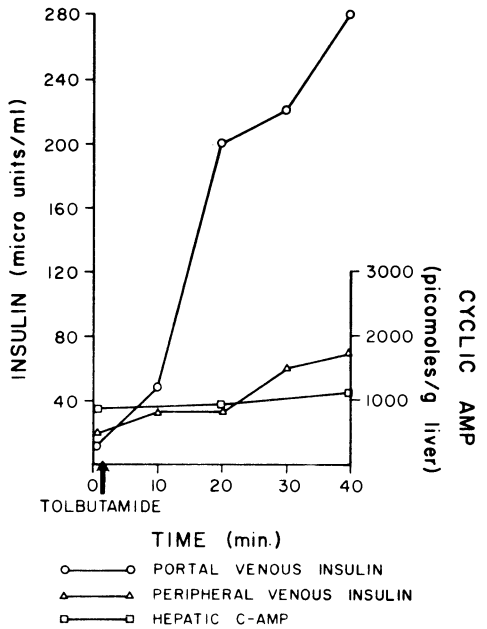

Fig. 5.— Changes in peripheral and portal venous insulin and hepatic cyclic 3', 5'-adenosine monophosphate, cyclic AMP, occurring in a normal dog infused with tolbutamide. Note that the peak insulin response in the portal blood occurred 25 to 40 minutes after infusion and that no significant alterations in hepatic cyclic 3', 5'-adenosine monophosphate were caused acutely by the tolbutamide itself.

powder was collected on a millipore filter. The filters were introduced into counting vials containing 20 milliliters of Albano and Francavilla's standard scintillation solution(1) and counted on a Picker Liquimat®. The results were expressed as counts per minute per gram wet weight of liver.

Total lipids and triglycerides. Total lipids were measured by a modification of the method of MacKenzie and his colleages(26), by which a tissue sample is homogenized in and extracted with a cold 1:1 ethyl alcohol:ethyl ether solution, washed, dried, and weighed.

The triglyceride fraction was extracted and weighed by a modification of MacKenzie's micromethod as derived from Barron and Hanahan(2), using silicic acid column chromatography to separate the triglyceride from the other lipid components. The column was first washed with 5 grams of 85:15 hexane:benzene. Then, the first two fractions of 4 grams each were eluted with a mixture of 97:3 hexane:ethyl ether and collected for triglyceride determinations. The total lipids and triglyceride fractions were expressed as milligrams per gram wet weight of liver.

Glucokinase. Glucose phosphorylation by glucokinase, Enzyme Commission Number 2.7.1.2., was assayed by the glucose-6-phosphate dehydrogenase spectrophotometric method of Salas and his colleagues(40). The results were expressed as micromoles of glucose phosphorylated per minute per gram wet weight of liver at room temperature.

Kinetic Studies of Cyclic 3', 5'-adenosine Monophosphate (Cyclic AMP)

Aminophylline test. To determine the rate of formation of liver cyclic 3', 5' - adenosine monophosphate, 13 dogs of groups 1 or 2 were submitted to an aminophylline infusion two months after their operation. As described by Robison and his group(38), the methylxanthines in appropriate dosages are essentially complete inhibitors of cyclic 3', 5' -adenosine monophosphate phosphodiesterase. Since Butcher and Sutherland (8) and Cheung (10) have shown this phosphodiesterase to be the principal enzyme involved in the catabolism of cyclic 3', 5' -adenosine monophosphate, inhibition of the enzyme with aminophylline allowed the quantitation of cyclic 3', 5' -adenosine monophosphate formation. A large dosage of aminophylline was used. This was given rapidly to permit prompt completion of the studies before the onset of delayed physiologic effects, such as aminophylline induced changes in insulin levels which might by themselves affect the metabolism of cyclic 3', 5' -adenosine monophosphate.

The dogs were anesthetized, and open biopsy specimens of the right and left lobes were taken for baseline levels. Then, 3 grams of aminophylline in 300 milliliters of 5 per cent dextrose in normal saline solution were infused by way of a peripheral vein at a constant rate during a six minute interval. Biopsy specimens of 100 to 200 milligrams in weight were removed from both sides of the liver at two, four, and six minutes after the infusion was begun, taking care to minimize blood loss. These were immediately frozen in liquid nitrogen and assayed for cyclic 3', 5' -adenosine monophosphate as described previously.

Tolbutamide-glucagon test. As reviewed by Exton and Park(13), the level of hepatic cyclic 3', 5' -adenosine monophosphate is, at least in part, under the control of endogenous insulin. To determine whether or not the livers of dogs remained responsive to changes in insulin level after a portal diversion procedure, a test was developed in which the cyclic 3', 5' -adenosine monophosphate response to a measured dosage of endogenous insulin was measured.

To generate a standard endogenous insulin response, a 40 milligram per kilogram dose of tolbutamide was given to normal dogs as a one minute infusion by way of a peripheral vein. With the insulin antibody method of Morgan and Lazarow(32), it was found that the peak insulin increase in the portal blood occurs 25 to 40 minutes after the infusion of tolbutamide (Fig. 5)

The tolbutamide itself did not cause major changes in hepatic cyclic 3', 5' -adenosine monophosphate (Fig. 5). Nevertheless, the levels of portal blood insulin generated by the test are known from other studies of Robison and his colleagues(38) to produce a reduction in the hepatic cyclic 3', 5' -adenosine monophosphate, even though these are so slight that they are difficult to measure by direct techniques. However, the effect of the insulin can be demonstrated by giving glucagon simultaneously as was demonstrated in the three control experiments (Fig. 6). Glucagon normally produces a many fold increase in liver cyclic 3', 5' -adenosine monophosphate when given by systemic vein, but when the liver received both endogenous insulin and exogenous glucagon simultaneously, the net effect was a more modest rise in cyclic 3', 5' -adenosine monophosphate (Fig. 6). In these control dogs, the right lobes of the liver had a normal blood supply whereas the left portal branch was occluded so that the left lobes did not directly receive any venous effluent from the pancreas.

The conditions of the tests in the 13 definitive dogs of groups 1 or 2 were as follows: the anesthetized dogs were maintained on lactated Ringer's solution while biopsy specimens of each side of the liver were taken. Tolbutamide, 40 milligrams per kilogram body weight, was given intravenously for one minute, following which an infusion of 5 grams per kilogram of dextrose was given over 25 minutes. Then, 1.40 gammas per kilogram body weight of glucagon were given in 300 milliliters of 5 per cent dextrose during eight minutes. Biopsy specimens were taken at two, four, six and eight minutes after the glucagon infusion was begun, frozen in liquid nitrogen, and assayed for the cyclic 3', 5' -adenosine monophosphate, as described previously.

Results

Partial Portacaval Transposition

Morphologic findings. Eighteen of the 33 experiments with unmodified split transposition were carried to completion with the proof of the patency of the venous channels after approximately two months. In 11 of these successful experiments, the inferior vena cava was anastomosed to the left portal branch (Fig. 1a), and in the other seven, the vena caval anastomosis was to the right branch (Fig. 1b).

The results which are partially summarized in Figure 7 confirmed and extended the observations previously reported from our laboratories(28,30). The liver lobes receiving splanchnic venous inflow gained weight after 2 ± 0.5 (standard deviation) months and had striking hypertrophy of the individual hepatocytes, whereas the lobes being perfused with venal caval blood shrank with a diminution of the liver cell size. Good histopathologic studies were obtained from 17 of the 18 livers. The liver lobules were larger on the splanchnic side in all of the 17 experiments, and lipid was less prominent in 12 of 17 and about equal in the other five. Glycogen, as judged with periodic acid-Schiff stained sections, often seem more prominent in the lobes supplied with splanchnic venous blood although this finding was inconstant. Consequently, heavy reliance was placed on the quantitative glycogen assays which will be described.

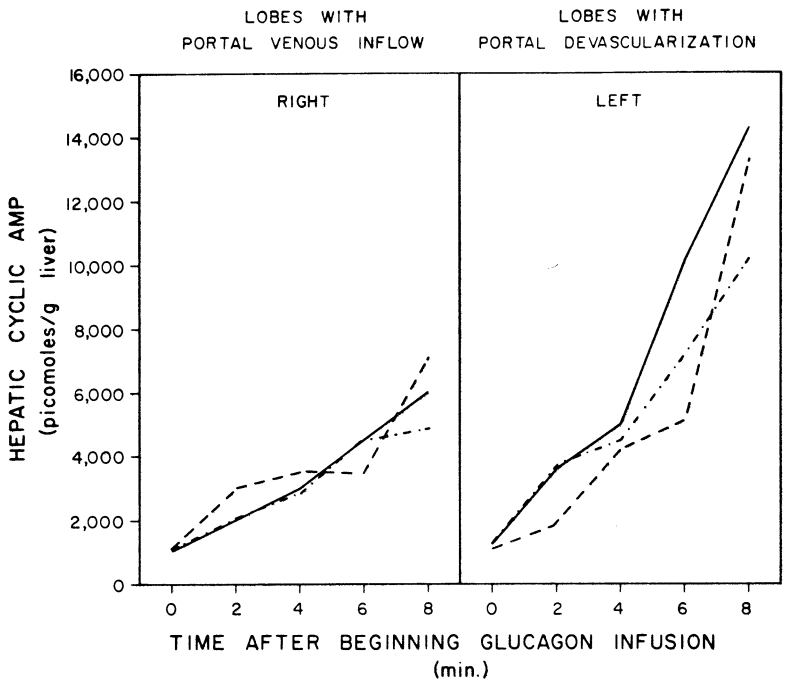

Fig. 6.— Results with the tolbutamide-glucagon test in three dogs from groups 1a or 1c in which there was clotting of the venous grafts. Consequently, the left branch of the portal vein transmitted no blood at all while the right lobes had a full splanchnic inflow. Note that the hepatic cyclic 3', 5'-adenosine monophosphate response to exogenous glucagon was considerably less in the lobes having a portal inflow compared wih the lobes suffering from portal devascularization. The relatively lower cyclic 3', 5'-adenosine monophosphate presumably represented a restraining insulin effect.

There was also evidence of hyperplasia on the side receiving splanchnic blood. This consisted of the presence of hepatic cell plates two or more cells thick, binucleate and trinucleate hepatocytes, increased numbers of bile ductules, and slightly increased mitosis count.

Of the eight attempts at split transposition with exclusion of the adrenal and renal blood, only one experiment was completely successful, that with vena caval flow to the right lobes (Fig. 1d). The morphologic effects of this procedure at nine weeks (Fig. 7) were comparable but slightly less extreme than with regular split transposition.

Biochemical concentrations. Beginning within four weeks and continuing until two months there were marked biochemical differences between the lobes according to the source of portal venous inflow (Table I). Hepatic tissue receiving splanchnic blood, whether this be on the right or left side, had significantly higher concentrations of glycogen and glucokinase relative to the lobes supplied by the vena caval blood. In contrast, the lobes supplied by the vena caval blood had significantly higher cyclic 3', 5' -adenosine monophosphate and active phosphorylase concentrations. The hepatic triglyceride concentration seemed to be increased by splanchnic venous inflow, but the observations were too few to permit statistical significance. The comparisons of protein concentration and protein synthesis were not strikingly different (Table I).

In the one successful experiment of group 1b (Fig. 1d) in which adrenal and renal venous blood was excluded from the vena caval supply to the right lobes, the chemical pattern was altered after nine weeks in a different way than in the dogs of group 1a. In the right and left lobes respectively, glycogen was 5.7 and 5.25 milligrams, glucokinase was 1.0 and 1.5 micromoles, active phosphorylase was 47.4 and 45.3 millimicromoles, and cyclic 3', 5' -adenosine monophosphate was 974 and 1,225 picomoles.

Splanchnic Flow Division

Forty experiments were attempted, and 12 were carried to completion. In six successful experiments, the pancreatic, splenic, and gastroduodenal blood passed to the right lobes (Fig. 2a), and in the other six the blood passed to the left lobes (Fig. 2b).

Morphologic findings. The effects of these alterations on the liver tissues after one to five and one-half months, 73 ± 53 (S. D.) days, are summarized in figure 8 and compared with the liver tissue from six normal dogs. The lobes which received the pancreatic, gastroduodenal, and splenic blood retained or increased their expected weight compared with the other lobes supplied by intestinal venous blood, and the individual hepatocytes in the favored lobes increased in size (Fig. 8). The latter hepatocyte and lobe size increases occurred whether the pancreatic, gastroduodenal, and splenic flow was to the left or right lobes. In addition to the hepatocyte size, complete histopathologic analysis was performed on all 12 livers. In nine of these 12 experiments, the liver lobules were obviously larger on the side supplied by the pancreatic-gastroduodenal-splenic venous effluent (Fig. 9). The degree of lipid deposition was less on this side in one experiment but about the same in the other 11.

There was also evidence of hyperplasia in the liver tissue supplied by blood from the pancreas, stomach, duodenum, and spleen (Fig. 9). This consisted of hepatic cell plates two or more cells thick, binucleate and trinucleate hepatocytes, and increased numbers of bile ductules. A slightly raised mitosis count was also found in those livers biopsied less than three months after splanchnic flow division.

Biochemical concentration. After 10.4 ± 7.6 (S. D.) weeks, range one to five and one-half months, the liver tissue provided with pancreatic-gastroduodenal-splenic blood contained higher concentrations of glycogen in spite of the fact that alimentary glucose was passing primarily to the contralateral hepatic lobes. These findings were statistically significant in the experiments of group 2b in which the pancreatic-gastroduodenal-splenic blood went to the left lobes (Table II). When this blood was directed to the right side in the dogs in group 2a, the right lobes behaved similarly, but the changes were short of statistical significance. Under both conditions, the glucokinase was elevated in parallel with the glycogen but not to

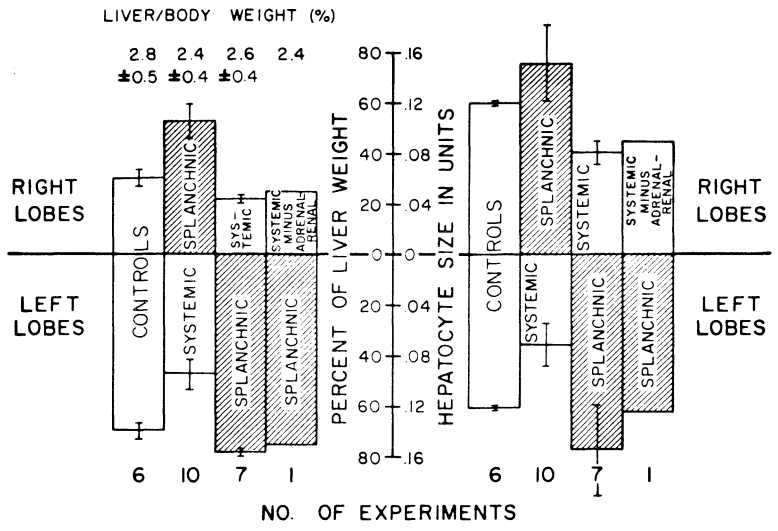

Fig. 7.— The morphologic consequences, after 2± 0.5 (S. D.) months in the dogs of group 1, of supplying parts of the liver with nonhepatic splanchnic venous blood, shaded areas, as compared with vena caval blood. Results are also given for six normal dogs. Note the gross weight gain and hepatocyte hypertrophy on the side perfused with splanchnic blood. Although the relative sizes of the lobes were altered according to the source of portal venous inflow, note that the ratio of the liver weight to total body weight was relatively unchanged. One standard deviation is depicted in the bar graphs and written out for the weight percentages.

TABLE I.—THE EFFECT IN SPLIT TRANSPOSITION EXPERIMENTS OF GROUP 1 OF PORTAL VENOUS INFLOW FROM THE SPLANCHNIC VERSUS THE INFERIOR VENA CAVAL BEDS

	Splanchnic flow to right lobes								*Splanchnic flow to left lobes*							
	One month				*Two months*				*One month*				*Two months*			
	No.	*Right*	*Left*	*p*	*No.*	*Right*	*Left*	*p*	*No.*	*Right*	*Left*	*p*	*No.*	*Right*	*Left*	*p*
Glycogen, mgm./gm. of liver																
Total	7	5.15 ±1.00	3.79 ±0.43	<0.02	11	3.74 ±1.16	2.26 ±1.08	<0.001	3	2.92 ±1.63	3.74 ±2.69	NS	7	2.45 ±0.70	4.66 ±0.76	<0.001
Trichloroacetic acid soluble	7	4.65 ±1.11	2.81 ±0.81	<0.02	11	3.00 ±1.03	1.59 ±0.88	<0.001					7	1.89 ±0.83	3.55 ±0.58	<0.01
Cyclic adenosine monophosphate, pico-moles/gm. of liver	7	1,050 ±159	1,638 ±284	<0.001	11	1,162 ±86	1,695 ±256	<0.001	3	1,276 ±185	906 ± 211	<0.1	6	1,718 ±96	1,363 ±267	<0.05
Phosphorylase, milli-micromoles/min./ mgm. of liver protein																
Total	7	111.5 ±11.3	*		11	123.1 ±17.7	*		3	*	123.5 ±17.0	NS	4	*	110.7 ±10.1	
Active	7	51.9 ±14.0	72.7 ±18.2	<0.1	11	54.8 ±11.8	76.0 ±13.2	<0.001	3	66.6 ±18.4	46.0 ±12.3	NS	4	72.3 ±4.3	44.8 ±6.1	<0.02
Glucokinase, micro-moles/gm./min.					9	3.04 ±1.14	1.73 ±0.67	<0.001					7	1.59 ±0.97	2.33 ±0.81	<0.01
Protein concentration, mgm./gm. of liver.	7	197.0 ±14.0	216.3 ±21.0	<0.05	11	205.4 ±21.4	211.7 ±20.9	NS	3	195.8 ±7.8	175.5 ±63.9	NS	7	188.5 ±17.6	184.2 ±15.6	NS
Protein synthesis, counts/min./gm. of liver	1	2,175	2,558		6	1,758 ±112	1,866 ±246	NS					2	2,016 ±378	2,021 ±243	NS
Triglycerides, mgm./ gm. of liver					3	16.8 ±3.0	11.6 ±1.6	<0.1								

Means, numbers of determinations, and standard deviations are given.
All p values compare the two liver sides.
*Within the limits of the analytic method used, the measurements of total phosphorylase activity were constant in a given experiment, no matter what the site of the liver biopsy. For this reason, these determinations are given only for the side of the liver receiving splanchnic blood.

TABLE II.—THE EFFECT IN SPLANCHNIC DIVISION EXPERIMENTS OF GROUP 2 OF PERFUSING THE LIVER WITH PANCREATIC DUODENAL SPLENIC VERSUS INTESTINAL BLOOD

	Splanchnic flow to right lobes								*Splanchnic flow to left lobes*							
	One month				*Two months*				*One month*				*Two months*			
	No.	*Right*	*Left*	*p*	*No.*	*Right*	*Left*	*p*	*No.*	*Right*	*Left*	*p*	*No.*	*Right*	*Left*	*p*
Glycogen, mgm./gm. of liver																
Total	5	5.28 ±1.26	5.26 ±1.47	NS	4	3.64 ±1.63	3.13 ±1.67	NS	4	3.31 ±1.90	4.51 ±1.91	<0.02	6	2.83 ±1.87	4.07 ±1.64	<0.01
Trichloroacetic acid soluble	5	4.11 ±1.05	4.03 ±1.05	NS	4	3.35 ±0.40	2.35 ±1.55	NS	4	2.93 ±1.90	3.70 ±2.05	<0.1	6	2.00 ±1.31	3.23 ±1.41	<0.001
Cyclic adenosine monophosphate picomoles/gm. of liver	5	1,276 ±102	1,258 ±76	NS	4	1,215 ±191	1,123 ±175	NS	4	725 ±63	1,052 ±394	NS	6	1,077 ±237	1,164 ±218	<0.1
Phosphorylase, millimicromoles/min./mgm. of liver protein																
Total	5	115.1 ±21.3	*		4	104.2 ±6.4	*		4	*	88.8 ±23.5		6	*	113.2 ±11.6	
Active	4	46.5 ±10.0	48.1 ±7.3	NS	4	52.0 ±6.3	50.4 ±6.3	NS	4	43.2 ±4.8	47.3 ±15.0	NS	6	49.2 ±7.9	48.5 ±7.3	NS
Glucokinase, micromoles/gm./min.					4	2.16 ±1.27	1.62 ±1.06	<0.1					4	2.07 ±1.13	2.67 ±0.88	NS
Protein concentration, mgm./gm. of liver.	5	188.3 ±35.9	190.7 ±29.9	NS	4	205.6 ±2.5	202.4 ±6.2	NS	4	179.0 ±7.0	210.0 ±19.8	<0.02	6	192.7 ±33.1	198.2 ±33.9	NS
Protein synthesis, counts/min./gm. of liver					4	2,696 ±394	2,753 ±358	NS	4	2,971 ±1,044	3,021 ±1,043	NS	3	2,340 ±970	2,509 ±341	NS

Means, number of determinations, and standard deviations are given.
All p values compare the two sides.
*Within the limits of the analytic method used, the measurements of total phosphorylase activity were constant in a given experiment, no matter what the site of the liver biopsy. For this reason, these determinations are given only for the side of the liver receiving splanchnic blood.

TABLE III.—THE EFFECT OF PORTACAVAL TRANSPOSITION (LEFT) AND PORTACAVAL TRANSPOSITION MINUS THE RENAL AND ADRENAL BLOOD (RIGHT) UPON GLYCOGEN CONCENTRATION AND CYCLIC 3′, 5′-ADENOSINE MONOPHOSPHATE

	Transposition				*Transposition minus renal, adrenal*			
	No.	*Preoperative*	*Postoperative*	*p*	*No.*	*Preoperative*	*Postoperative*	*p*
Glycogen, mgm./gm. of liver								
Total	5	3.82±0.53	2.10±1.04	<0.02	2	4.08±1.12	2.27±0.40	NS
Trichloroacetic acid soluble	5	3.13±0.42	1.70±0.83	<0.001	2	2.99±0.55	1.89±0.31	NS
Cyclic adenosine monophosphate, picomoles/gm. of liver	5	(1107)*	1,729±215	<0.01	2	(1107)*	1,701±46	<0.05

The experiments are group 3a and 3b.
All p values compare the preoperative and postoperative determinations.
*This "normal" for cyclic 3′, 5′-adenosine monophosphate was derived by taking the mean of a series of cyclic 3′, 5′-adenosine monophosphate determinations in biopsy specimens taken from canine livers having a normal portal blood supply.

a statistically significant degree (Table II).

Cyclic 3', 5' -adenosine monophosphate, active phosphorylase, protein concentration, and protein synthesis did not conform to a consistent pattern.

Total Portacaval Transposition

The five dogs with successful conventional transposition (Fig. 3a) underwent follow-up study for 10.2 ± 2.0 (S. D.) weeks, and the dogs with transposition minus adrenal and renal blood were each studied for two months. At autopsy, the per cent of total liver to total body weight was 1.8 and 1.9 per cent in the respective groups.

The dogs with standard as well as those with modified portacaval transposition had hepatic deglycogenation and striking elevations in cyclic 3', 5' -adenosine monophosphate (Table III).

Kinetic Studies

Partial portacaval transposition. In four of five dogs of group 1a (Fig. 1a or b), the rate of cyclic 3', 5'-adenosine monophosphate formation, as unmasked by the aminophylline test, was greater in the liver tissue receiving vena caval blood than in the contralateral lobes receiving splanchnic venous blood (Fig. 10).

In the single successful experiment in which partial transposition was with vena caval blood minus adrenal and renal inflow (Fig. 1d), the cyclic 3', 5' -adenosine monophosphate response showed a different pattern in that the side of the liver supplied with vena caval blood had a much slower rate of cyclic 3', 5' -adenosine monophosphate accumulation than the lobes receiving splanchnic blood (Fig. 11). These results were similar to those in three control experiments in which one side of the liver did not have portal vein inflow at all due to clotting. This side accumulated cyclic 3', 5' -

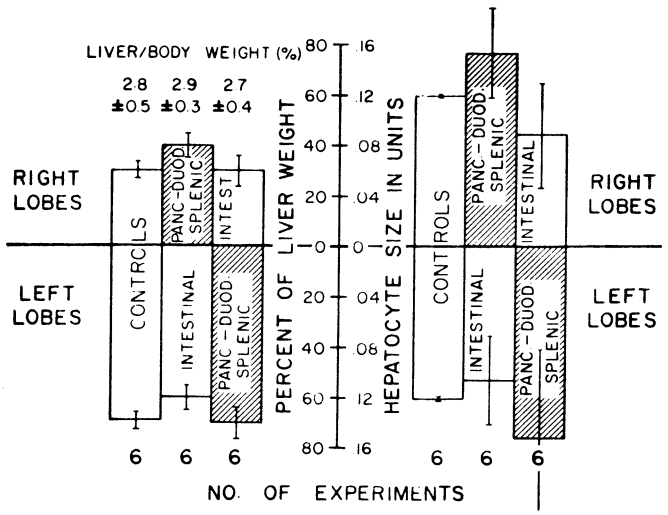

Fig. 8.— The morphologic consequences of splanchnic venous flow division in the dogs in group 2 compared with normal dogs after 28 to 173 days, average 73. The liver fractions which were perfused with venous blood from the pancreatic, gastroduodenal, and splenic areas are shaded. Note that these portions gained weight and underwent an increase in hepatocyte size relative to the other side while the total liver weight to body weight ratios were little altered. One standard deviation is depicted graphically on the bar graphs and written out for the weight percentages.

adenosine monophosphate much more slowly than in the fully vascularized lobes (Fig. 12).

With the tolbutamide-glucagon test, the lobes receiving nonhepatic splanchnic blood showed a response similar to that of the control dogs, whereas the contralateral lobes supplied with the supra-adrenal vena caval blood showed a rapid accumulation of cyclic 3', 5' -adenosine monophosphate (Fig. 13).

Splanchnic flow division. With the aminophylline infusion test, it was found in four experiments that the lobes receiving splenic-gastroduodenal-pancreatic blood had a more rapid rate of cyclic 3', 5' -adenosine monophosphate synthesis than the contralateral lobes supplied with intestinal blood (Fig. 14).

However, the lobes with splenic-gastroduodenal-pancreatic venous inflow were found by the tolbutamide test to be under insulin control in that three of five experiments showed a much slower rate of cyclic 3', 5' -adenosine monophosphate accumulation in response to exogenous glucagon than in the lobes receiving mesenteric blood (Fig. 15).

Discussion

Little doubt remains that there are substances in splanchnic venous as opposed to systemic venous blood that are important for the maintenance of hepatic structure and function. The first steps by which this concept was developed and validated have already been mentioned. The extent of present day acceptance of the concept can be appreciated by the fact that Fisher and Lee and their associates(15-17, 24), who until recently were the most outspoken critics of the hepatotrophic hypothesis, have lately supported the idea. By adapting the double liver fragment principle introduced during investigation of auxiliary liver transplantation in our laboratory(28, 30, 51), they and their colleagues have added convincing evidence of their own supporting the hepatotrophic theory(9, 18, 25).

The advantage of the various double or split liver preparations for the investigation of hepatotrophic mechanisms is that the hepatotrophic factors are apparently exhausted by exposure to one hepatic fragment and, therefore, are unavailable to other competing liver tissue if the latter is endowed with a blood supply deficient in such substances. With a single liver, as historically was used in the studies of Eck fistula or portacaval transposition, the unmasking and precise study of splanchnic hepatotrophic effects was difficult or impossible after the portal by-pass procedures since biologically active substances in the diverted spanchnic venous effluent were presumably returned to the liver by way of the systemic blood although in diluted concentrations. Parenthetically, a recirculation effect could account for the superior liver state of dogs with Child's transposition compared with animals with Eck fistula since the quantity of diluted hepatotrophic substances reaching the liver would be proportional to the total hepatic blood flow which is more than twice as great with transposition than with Eck fistula. Then, the classical error in interpretation followed, namely to assume the quantity of blood flow was infinitely more important than the quality of the blood in maintaining hepatic structure and function.

Conceding the qualitative specialness of portal venous blood, the experiments in this study were designed to answer two sets of additional questions The first concerned the source and the nature of hepatotrophic factors in splanchnic venous blood. The second was involved with the mechanism of action of this factor or complex of factors.

The origin of the hepatotrophic factors was determined from studies of the morphologic and biochemical changes induced in the liver by modifications of the portal venous inflow. These were of three types: total portacaval transposition, partial portacaval transposition, and splanchnic flow division.

The observations of Marchioro and his associates(28, 30) were first confirmed in experiments with partial transposition. Within four to eight weeks, liver lobes supplied with splanchnic venous inflow had hypertrophic glycogen-rich hepatocytes which in addition often had findings of hyperplasia. In contrast, the other lobes of the liver supplied by vena caval blood underwent involution changes. The hepatocytes became smaller, and the glycogen concentration decreased.

Next, surgical techniques were used that partitioned the splanchnic flow. The major part of the hepatotrophic influence as manifested both by morphologic and biochemical criteria was unequivocally shown to be in the blood returning from the pancreas, proximal part of the duodenum, stomach, and spleen. In contrast, the other hepatic lobes fed by nutritionally rich venous blood from the small intestine underwent involutional changes, including atrophy (Fig. 16) and deglycogenation nearly as profound as if a vena caval supply had been used.

For several years, there has been good reason to suspect that the upper splanchnic organ complex and specifically the pancreas was the source of

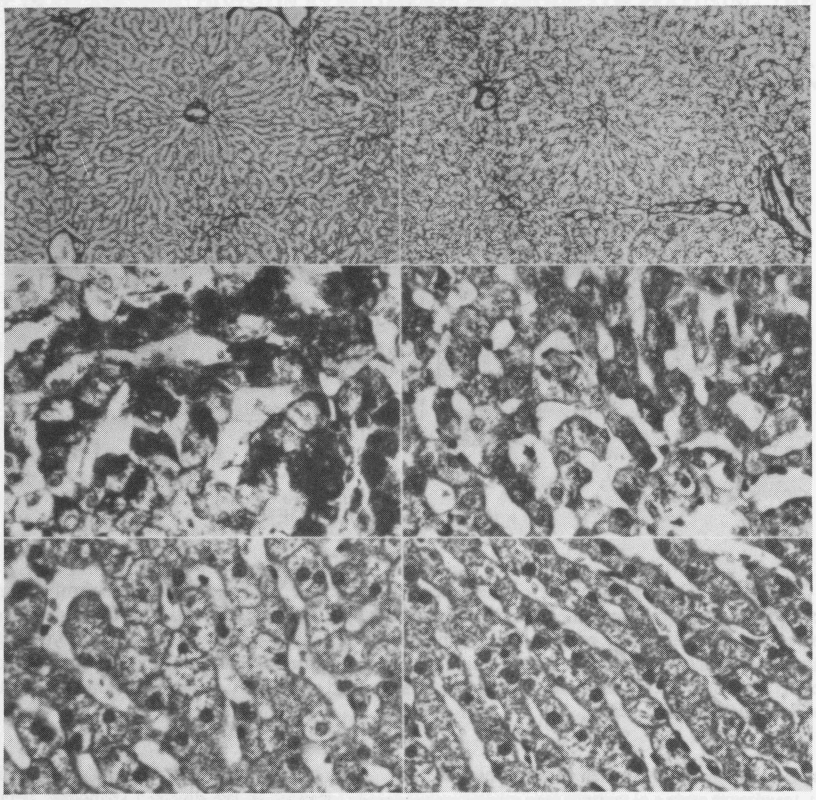

Fig. 9.— Photomicrographs of sections from a liver which had been subjected to splanchnic flow division. The part of the liver supplied by the pancreaticogastroduodenal-splenic blood, panels on left, shows enlargements of the liver lobules, abundant glycogen, and hypertrophy and hyperplasia of hepatocytes when compared with the part supplied by intestinal blood, panels on right. Upper, Reticulin stain, X 10. Middle, Periodic acid-Schiff, X 87. Lower, Hematoxylin and eosin, X 87.

the hepatotrophic factors. In the studies of the blood flow requirements for auxiliary hepatic transplantation by Marchioro and his colleagues (29), atrophy of the native liver could be minimized by provision of pancreatic-gastroduodenal-splenic blood even though all the rest of the splanchnic venous blood went to a co-existing liver homograft. Pouyet and his associates (34) came to the same general conclusion with carefully documented splanchnic division experiments similar to those in group 2 of this study. The correctness of these observations was later confirmed in a beagle homotransplantation model used by Ranson and his associates (37). In all these studies as well as in those reported herein, a contribution to the hepatotrophic support of the liver could hypothetically have been made from the stomach, duodenum, or spleen although some of Pouyet's experiments essentially ruled out the spleen and stomach. Nevertheless, it was logical as Price and his associates (35) have suggested to look for a hormonal explanation. This was done in the present study with a series of biochemical evaluations.

The first step was to analyze the hepatic changes caused by the classical portacaval transposition of Child and his associates (11), a procedure which profoundly deglycogenates the liver (43, 52) as was confirmed in the present study, deprives it of access to pancreatic hormones until after recirculation by way of the hepatic artery or systemic venous blood, and subjects the whole organ more or less continuously to endogenous epinephrine. Since Sutherland and Rall (53) and Murad and his associates (33) have shown that epinephrine works by the activation of adenyl cyclase and the consequent formation of cyclic 3', 5'-adenosine monophosphate, the finding of elevated concentrations of cyclic 3', 5'-adenosine monophosphate in the total transposition livers of the dogs in group 3a was consistent with the concept that epinephrine was being excreted in large enough quantities to play a significant role in the deglycogenation. Nevertheless, proof that direct hepatic perfusion by endogenous epinephrine was not the only factor promoting these changes was provided by the experiments of group 3b in which transposition minus the adrenal and renal blood was successfully carried out in two dogs. Falls in hepatic glycogen concentration and rises in cyclic 3', 5'-adenosine monophosphate occurred of almost the same magnitude as in the standard transposition. Here, it might be suggested that adrenal secretions which by-passed the liver were recirculated to the organ after passing through the cardiac mixing chamber and even the peripheral capillary beds. Obvious atrophy was not produced in these transposition livers with or without direct provision of adrenal venous blood.

In the split transposition experiments of group 1a, increases in cyclic 3', 5'-adenosine monophosphate similar to those caused by a standard transposition were found in the deglycogenated and atrophic lobes of the liver receiving supra-adrenal vena caval blood as compared with the lobes supplied with splanchnic venous blood. In these experiments, the increases of activated glycogen phosphorylase and the decreases in triglyceride concentration in the lobes perfused with vena caval blood were consistent with the metabolic consequences of epinephrine infusion and increased cyclic 3', 5'-adenosine monophosphate, as summarized by Himms-Hagen (22). The fact that the concentration of endogenous epinephrine was physiologically significant was also suggested by some of the portal angiograms in the split transposition experiments which showed relative vasoconstriction in the lobes being perfused by supra-adrenal vena caval blood. Thus, it was not surprising in the one successful split transposition experiment in group 1b that exclusion of the adrenal and renal venous blood from the lobes supplied by the inferior vena cava curtailed both the rise in cyclic 3', 5'-adenosine monophosphate and the fall of glycogen concentration although atrophy was not thereby prevented. These last findings were in contrast to the observations discussed in the preceding paragraph in the livers of two dogs that had total transposition minus the

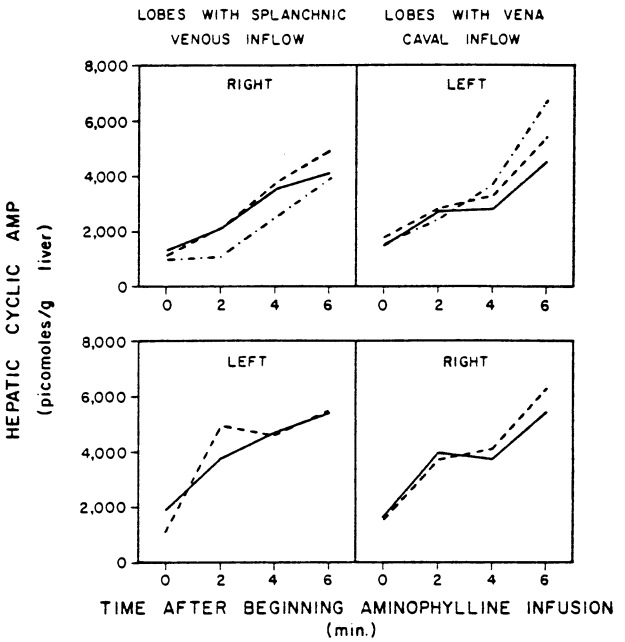

Fig. 10.— Aminophylline infusion tests in five dogs with partial portacaval transposition. In all but one, the rate of formation of cyclic 3', 5'-adenosine monophosphate was greater in the lobes deriving their portal inflow from the inferior vena cava although these differences were usually not obvious until several minutes had gone by.

adrenal and renal blood. The apparent disparities indicated once more that influences other than the adrenal secretions were of importance in regulating cyclic 3', 5'-adenosine monophosphate levels and in determining atrophy or hypertrophy. They focused attention upon the crucial role of the pancreas.

Such a pancreatic role may be assumed to be due to the interactions of glucagon and insulin for which cyclic 3', 5'-adenosine monophosphate also represnts a secondary messenger system. Like epinephrine, pancreatic glucagon increases cyclic 3', 5'-adenosine monophosphate as demonstrated by the classical studies of Sutherland and Rall (53), setting in motion multiple chemical processes. Bergen (3) and Weintraub (61) and their associates described the resulting glycogenolysis. Gluconeogenesis has been documented by Exton and Park (14), lipolysis by Butcher and his associates (6), and ketogenesis by Menahan and Wieland (31) as well as by Exton and his group (12).

As reviewed by Exton and Park (13), Sutherland and Robison (54), and Robison and his colleagues (38), insulin promotes many converse metabolic events by depression of the basal level of cyclic 3', 5'-adenosine monophosphate, thus qualifiying as an anabolic hormone. Besides aiding glycogen synthesis by the cyclic 3', 5'-adenosine monophosphate mechanism, Salas and his co-authors (40) have shown that insulin supports glycogen metabolism by increasing hepatic glucokinase, and Larner (23) has demonstrated activation of glucose transferase. Consequently, in the liver partition experiments of groups 1 and 2, it was not surprising that glucokinase levels were elevated on the side of pancreas inflow and reduced on the side receiving either vena caval or intestinal flow. Butcher (7) and Robison (38) and their colleagues have shown that insulin regulates lipid synthesis by inactivating lipolytic enzymes through a lowering of cyclic 3', 5'-adenosine monophosphate levels. Insulin also controls protein synthesis by a mechanism that is not understood.

The fact that glucagon and insulin have partially cancelling effects helps explain why the cyclic 3', 5'-adenosine monophosphate levels in liver lobes with an inflow of pancreatic venous blood tended neither to be very high nor very low. In turn, it helps explain why in the experiments of group 2 significant differences could not be demonstrated between the cyclic 3', 5'-adenosine monophosphate levels of liver lobes receiving pancreatic venous blood versus those receiving the hormone-poor effluent from the small intestine.

The aforementioned biochemical studies provided insight about how

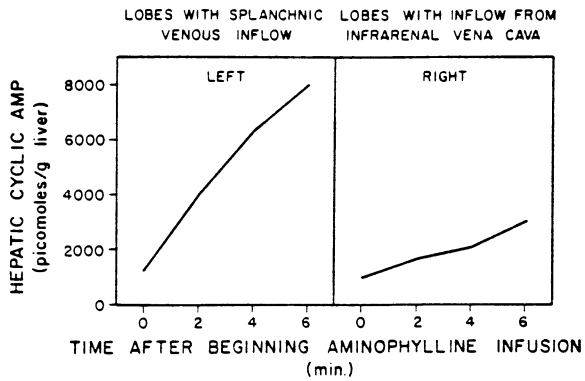

Fig. 11.— Rate of cyclic 3', 5'-adenosine monophosphate formation, as measured by the aminophylline infusion test, in the single successful experiment of group 1d.

various hormones could affect the liver. However, since cyclic 3', 5'-adenosine monophosphate is destroyed so rapidly, mainly by the enzyme cyclic 3', 5'-adenosine monophosphate phosphodiesterase, accurate evaluation of the rate of its production by the simple measures of its tissue concentration was not possible. Consequently, in several special experiments on the dogs of group 1, aminophylline was used to block the phosphodiesterase. The considerable extent to which the rate of cyclic 3', 5'-adenosine monophosphate production was increased in the hepatic tissue exposed to adrenal venous blood could then be determined.

The same kind of information was obtained in the livers of group 2 dogs in which neither hepatic side received adrenal venous blood directly. The aminophylline test uncovered a striking difference between the two sets of liver lobes. The rate of cyclic 3', 5'-adenosine monophosphate production was retarded in the liver lobes receiving blood from the intestine but was essentially normal in the lobes receiving pancreatic-gastroduodenal-splenic blood. It might be suggested that the lobes receiving blood from the intestines in such experiments should have higher cyclic 3', 5'-adenosine monophosphate levels if there were an unopposed presence of intestinal glucagon. However, Unger (58) and Valverde (59) and their associates have shown that this substance which has some physiologic qualities similar to pancreatic glucagon and which in addition cross reacts with antiglucagon antibodies does not affect the level of hepatic cyclic 3', 5'-adenosine monophosphate when injected into the portal circulation of the dog or into the perfusate nourishing an isolated rat liver.

The role of insulin in controlling the level of cyclic 3', 5'-adenosine monophosphate was further documented by the tolbutamide-glucagon

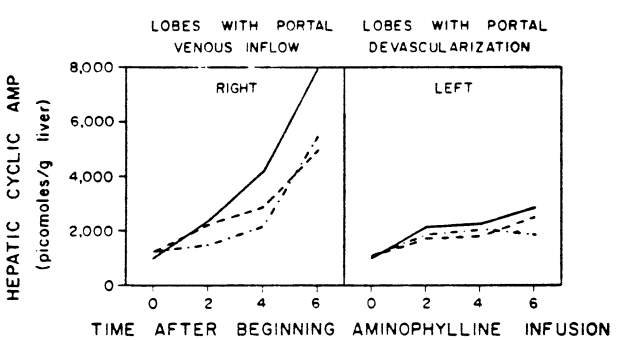

Fig. 12.— Aminophylline infusion tests in three dogs taken from groups 1a or 1c in which the left portal branch clotted, leaving these lobes supplied only with arterial blood, whereas the right lobes received both arterial and splanchnic venous blood. Note that the rate of synthesis of hepatic cyclic 3', 5'-adenosine monophosphate was greater in the right lobes having a portal inflow than in the left lobes suffering from total portal devascularization. The time units are in minutes.

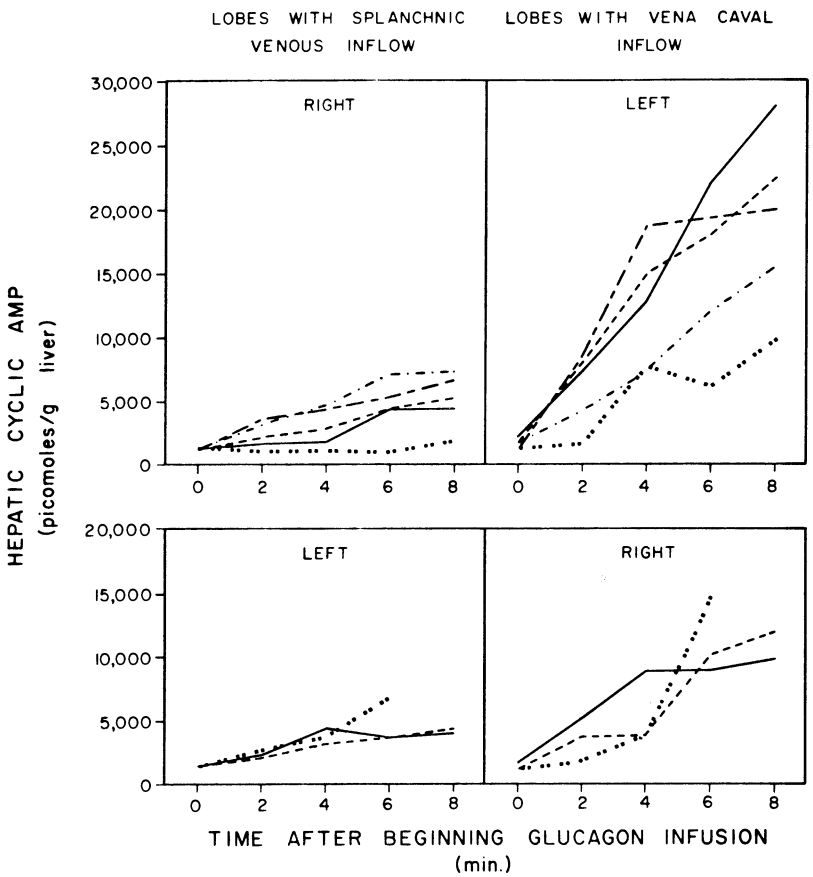

Fig. 13.— Results of tolbutamide-glucagon tests in eight dogs with partial portacaval transposition, demonstrating the effect of endogenous insulin in the lobes receiving splanchnic venous blood. These insulin-controlled lobes had a restrained cyclic 3', 5'-adenosine monophosphate response to the exogenous glucagon whereas the response in the other lobes was uninhibited.

infusion test. In this experiment, measurable increases in endogenous portal insulin were caused by an injection of tolbutamide. This was followed by the intravenous administration of exogenous glucagon. In all the experiments performed, there was a striking difference in the production of cyclic 3', 5'-adenosine monophosphate between the liver sides according to the type of blood supply, indicating once again a profound metabolic dissociation. Hepatic lobes perfused with pancreatic blood and, thus, under insulin influence had modest or no rises in cyclic 3', 5'-adenosine monophosphate in spite of the exogenous glucagon whereas the other lobes had uninhibited increases.

The data accumulated in this study leave little doubt that the previously nebulous hepatotrophic factors in splanchnic venous blood are hormones and that the major anabolic hormone is insulin counterbalanced to an unknown degree by the opposing effects of glucagon and possibly epinephrine as well. The interrelationship of these hormones determines the chemical environment of the liver thereby influencing its function and structure. In most of the experiments herein reported, the competing liver fragment preparation developed during research on auxiliary hepatic transplantation was the experimental tool whereby the metabolic dissociation and morphologic changes caused by different hormone exposure easily could be exposed and examined. But the findings in this special experimental model have major implications in normal as well as abnormal hepatic physiology.

For example, the reason for the liver atrophy caused in animals by the performance of Eck fistula obviously involves hormone deprivation. Explanations of a similar kind can be easily formulated for other portoprival states, including those caused in man by portacaval anastomosis of a portal vein which has significant residual hepatopetal flow. Why portal diversion procedures have been of such great value for children with certain of the glycogen storage diseases (52) becomes evident. Even in attempts at liver preservation, it may be suggested that long term conservation by perfusion is not apt to be successful unless the chemical and hormonal constituency of the perfusate is made to simulate at least some of the features of portal blood.

Although numerous other examples could be cited of the potential importance of hormonal mechanisms affecting the liver, attention will be focused only upon the virtual certainty that such hormonal factors are central to an understanding of hepatic regeneration. The way in which the portal hormone constituents can direct liver regeneration will require further detailed study from which clarification of some confusing past claims should emerge. A number of authorities on hepatic regeneration, including Sigel (48), Fisher (18), and Price (35) and their colleagues, have suggested that hypertrophy and hyperplasia are dissociated phenomena during regeneration. Price and his associates (35) have stated that there actually is a reciprocal relationship between hypertrophy and hyperplasia. The latter authors believe that hypertrophy is controlled by endogenous glucagon, and that hyperplasia is determined by some factor released by the liver itself.

Almost all the experiments purporting to show a dissociation between hepatocyte hypertrophy and hyperplasia have involved studies of deoxyribonucleic acid or ribonucleic acid synthesis or cell mitosis counts within a few hours or days after a major traumatic procedure. In our own studies which permitted chronic observations, hypertrophy and hyperplasia occurred together, although not necessarily in absolute parallel, in hepatic fragments supplied by either total splanchnic blood or blood from organs in the upper part of the abdomen whereas atrophy and the absence of hyperplasia were found in liver tissue deprived of the appropriate splanchnic influence. These observations indicate that hypertrophy and

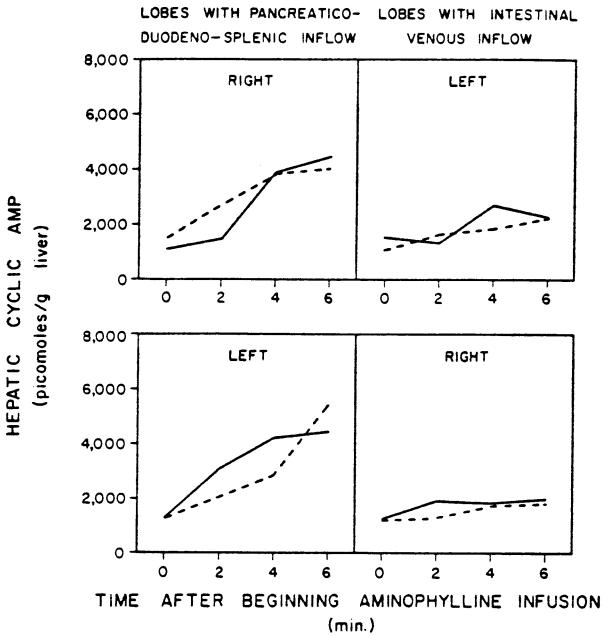

Fig. 14.— Aminophylline infusion tests two months after splanchnic flow division in four dogs. Cyclic 3', 5'-adenosine monophosphate synthesis was much more rapid in the hepatic lobes receiving pancreatic-gastroduodenal-splenic blood than in the contralateral lobes supplied with intestinal venous effluent.

hyperplasia are associated rather than dissociated, a point of view more in accord with the majority of regeneration studies which were recently reviewed by Bucher (5).

The findings are also consistent with the hypothesis that the hormonal hepatotrophic substances which have been the subject of this article are, in fact, the essential control factors of or at least play a vital role in hepatic regeneration.

Summary

The origin of hepatotrophic factors in splanchnic venous blood was investigated by modifying the portal venous inflow to different parts of the canine liver while leaving the arterial blood supply and biliary drainage intact.

In one variety of experiment, termed partial transposition, the liver portion perfused with the total splanchnic venous blood underwent weight gain and hepatocyte hypertrophy, hyperplasia, and glycogenation compared with the portion perfused with venous blood from the hindquarters, kidneys, and adrenal glands, but the combined weight of the total liver remained constant in spite of the rapidly evolving regional disproportions. The lobar changes were well developed within one to two months. At this time, the hepatic lobes supplied with splanchnic venous blood had higher concentrations of glucokinase and lower concentrations of cyclic 3', 5'-adenosine monophosphate and active phosphorylase than the lobes receiving hindlimb and adrenorenal venous blood, indicating that the biochemical environment of the different liver regions was drastically different by virtue of being under specific hormonal control.

The dissociation was even more dramatically illustrated by dynamic studies in which the destruction of cyclic 3', 5'-adenosine monophosphate by phosphodiesterase was blocked with aminophylline thereby permitting estimation of the rate of formation of cyclic 3', 5'-adenosine mono-

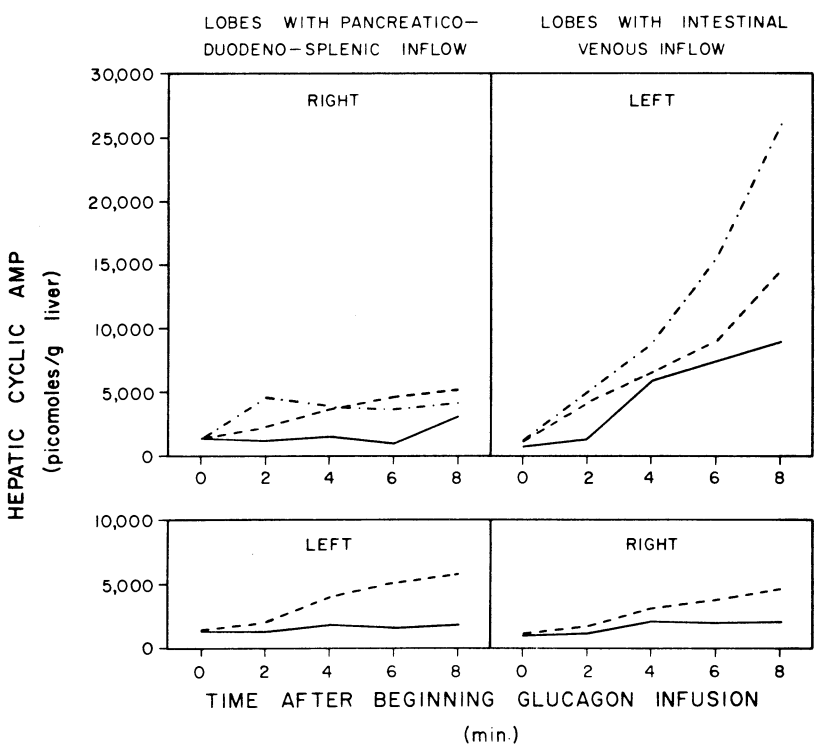

Fig. 15.— Tolbutamide-glucagon infusion tests in five dogs two months after splanchnic flow division. In the two experiments in which pancreatic-gastroduodenal splenic blood passed to the left lobes, group 2b, there were no significant differences in the hepatic cyclic 3', 5'-adenosine monophosphate concentrations on the two sides of the liver. However, in the three dogs of group 2a, top, there was a runaway response in the lobes receiving intestinal venous blood compared with a restrained response in the lobes nourished by pancreatic-gastroduodenal-splenic venous blood.

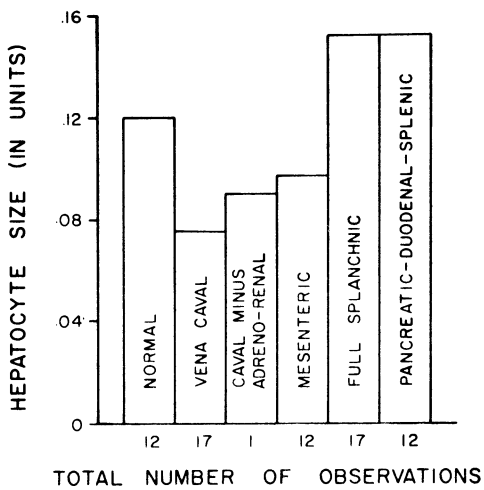

Fig. 16.— Summary from the different experiments depicted in Figures 1 and 2 shows the influence of the type of portal inflow upon hepatocyte size. The data are taken from Figures 7 and 8 and are compared with values obtained from the livers of normal dogs. Note that the presence of hypertrophy or atrophy was almost exclusively under the control of pancreatic-gastroduodenal-splenic blood and that the addition or subtraction of blood from other sources was of little further consequence.

phosphate. In addition, the modifying effect of tolbutamide-induced endogenous insulin upon exogenously administered glucagon was evaluated by serial determinations of cyclic 3', 5'-adenosine monophosphate. These investigations with the aminophylline and tolbutamide-glucagon tests demonstrated the anabolic role of insulin and the opposing roles of both glucagon and epinephrine in contributing to liver homeostasis. Epinephrine and glucagon caused striking increases in cyclic 3', 5'-adenosine monophosphate, and insulin had the converse effect.

Another type of preparation involving partition of the splanchnic venous blood between the liver portions was termed splanchnic flow division. The substances responsible for the hepatic hypertrophy, hyperplasia, glycogenation, and weight gain were shown to emanate mainly, if not virtually exclusively, from the pancreatic-gastroduodenal-splenic venous drainage. In contrast, intestinal nutritional substrate and hormones from the intestine or adrenal gland were not profoundly influential in either promoting or preventing the morphologic or glycogen concentration changes. The concentrations of cyclic 3', 5'-adenosine monophosphate, phosphorylase, and glucokinase in the two sides of the liver did not follow as distinctive a pattern as in the partial transposition experiments. However, the aminophylline and tolbutamide-glucagon tests revealed the same type of major dissociation of cyclic 3', 5'-adenosine monophosphate as with the partial transpositions. Particularly impressive was the way in which trace doses of tolbutamide-induced endogenous insulin on the side nourished by pancreatic venous blood restrained the cyclic 3', 5'-adenosine monophosphate response to exogenous glucagon, whereas the other liver fragment which was not so covered by insulin had completely uninhibited rises in cyclic 3', 5'-adenosine monophosphate.

The conclusion from these experiments is that the hepatotrophic factors previously reported from our laboratories and by other investigators to be in splanchnic venous blood are pancreatic hormones and specifically insulin and glucagon. Of these, insulin is anabolic and glucagon is mainly catabolic but not exclusively so, since glucagon also has the anabolic effect of stimulating gluconeogenesis. The insulin-glucagon relationship and the interrelationship of these hormones to others, such as epinephrine, in the moment to moment regulation of nutrient and hepatic homeostasis is a central fact of liver physiology that should reconcile a number of previously divergent opinions about portoprival syndromes, mechanisms of hepatic atrophy and hyperplasia, and the control of liver regeneration.

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These experiments directly tested the hypothesis that insulin was the principal hepatotrophic substance by evaluating the effect of insulin infusion into one of the main portal branches in the hilum after complete portacaval shunt. The liver fraction infused for four days with insulin retained cell size and morphologic integrity compared to the contralateral insulinoprival fragment which atrophied. Remarkable differences also were noted in hepatocyte proliferation.

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Effects of insulin, glucagon, and insulin/glucagon infusions on liver morphology and cell division after complete portacaval shunt in dogs

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Summary

Insulin, glucagon, and insulin/glucagon mixtures have been infused for four days into the left portal vein of dogs after portacaval shunt. In the left but not the right liver lobes, insulin alone reduced atrophy, preserved hepatocyte ultrastructure, and trebled cell renewal. Glucagon alone had no effect. In small doses glucagon alone did not potentiate the action of insulin and in large doses it may have reduced the insulin benefit. These studies explain the development of the previous mysterious Eck fistula syndrome, provide clues about in-vivo cell growth control by hormones, and suggest new lines of inquiry about the pathogenesis and/or treatment of several human disease processes.

Introduction

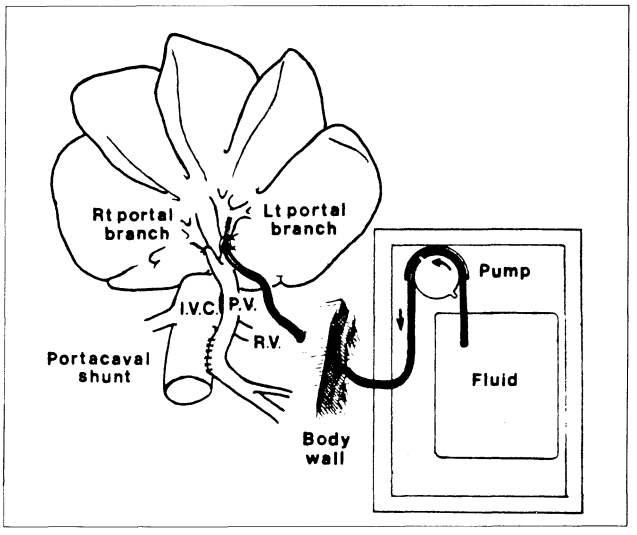
A completely diverting portacaval shunt (Eck's fistula) profoundly alters the structure and function of the liver in several species.[1] Until recently, these changes were thought[2-6] in what has been termed the "flow hypothesis" to be caused by and be proportionate to the reduction in total hepatic blood-flow which the procedure causes.[4] We have suggested instead that disruption of normal endogenous insulin delivery to the liver was mainly responsible[7-9] and in preliminary communication we showed that infusion of commercial insulin into the tied-off central portal vein of dogs prevented most of the acute atrophic changes that are ordinarily very advanced by light microscopy within four days after portacaval shunt.[10]

In this investigation, more complete studies were performed in dogs of hormone infusions after Eck's fistula. Insulin, glucagon, and combinations of these two agents in various doses were tested for their effect upon the morphology and division-rates of the hepatocytes.

Methods

Anæsthesia for operation and later killing with sodium pentobarbitone supplemented with phencyclidine hydrochloride ('Sernylan') and succinylcholine chloride ('Anectine'). Large side-to-side portacaval shunts were constructed with excision of ellipses from both portal vein and the inferior vena cava. The shunts were made completely diverting by individually ligating the main right and left portal trunks. Frequent morning blood-sugars were determined during the period of study by means of an 'AutoAnalyzer'.

The experimental groups of mongrel dogs are shown in table I. In groups 2-9, the tip of a fine infusion catheter was placed into the tied-off left portal branch and led through the body wall and through a long



Experimental arrangement in groups 2-9.
I.V.C. = inferior vena cava. P.V.= portal vein. R. V. = renal vein.

TABLE I—EXPERIMENTAL GROUPS AND HORMONE DOSES

Group no.	No. of experiments	Dog weights (kg) (mean±S.D.)	Type of portal infusion*	Insulin dose (units/kg/day) (mean±S.D.)	Glucagon dose (mg/kg/day) (mean±S.D.)	Cell size units (mean±S.D.)			P values†
						LO	L4	R4	L4 *vs* R4
1	11	18·0±5·3	No treatment	0	0	0·197±0·04	0·108±0·02	0·108±0·02	N.S.
2	6	18·3±5·1	Heparinised saline	0	0	0·200±0·04	0·104±0·02	0·104±0·01	N.S.
3	13	17·8±3·4	Large dose insulin	0·43±0·05	0	0·189±0·03	0·160±0·02	0·100±0·02	<0·001
4	4	17·5±3·5	Small dose insulin	0·16±0·11	0	0·194±0·04	0·143±0·02	0·094±0·01	<0·05
5	8	17 ±2·9	Purified insulin	0·42±0·08	0	0·196±0·04	0·158±0·01	0·095±0·02	<0·001
6	8	18·9±2·9	Small dose glucagon	0	0·0053±0·0011	0·189±0·01	0·103±0·01	0·103±0·01	N.S.
7	6	17·5±2·7	Large dose glucagon	0	0·60 ±0·10	0·165±0·03	0·085±0·01	0·082±0·01	N.S.
8	7	15 ±2·4	2/1 insulin/glucagon	0·45±0·03	0·0053±0·0005	0·211±0·05	0·156±0·05	0·094±0·02	<0·05
9	7	17·6±4·1	2/100 insulin/glucagon	0·42±0·01	0·50 ±0·02	0·215±0·04	0·114±0·03	0·073±0·02	<0·05

*All insulin was commerical regular insulin, except in group 5. †Comparisons were by Student's *t* test.

subcutaneous tunnel to a small calibrated finger pump that was incorporated into a light body cast (see accompanying figure). The infusion volumes never exceeded 25 ml per day. Regular commercial insulin from beef and pork pancreas was used for much of the work. To rule out artefacts from contaminants, research-quality bovine insulin prepared by the Eli Lilly Company (Indianapolis) was used in group 5 (lot no. 24C-3130). The glucagon which was pure (Lilly lot no. 95C-0314) came from beef and pork pancreas. The small and large glucagon infusion doses in the glucagon-alone groups 6 and 7 respectively would have provided approximately a 2/1 or 2/100 insulin/glucagon molar ratio had insulin been given in the amounts used in groups 3 and 5. A 2/1 ratio is in the physiologic range.[11]

Biopsy specimens were obtained from a left lobe at the time of portacaval shunt (LO), and from left and right lobes (L4 and R4) at the time of killing. About two hours before killing, 63 of the 70 dogs were given 4.5 mC of (CH_3-^{3}H) thymidine by the intravenous route. The specific activity of the thymidine was 6.4 C per mmol. Because the same dose was always used without regard to animal size, the dose spectrum per kilogrammme per body weight was broad, ranging from 0.16 to 0.48 mC. Shunt patency and catheter position were always verified at necropsy.

After fixation in formalin, frozen sections were cut from the samples of liver tissue and stained with sudan-4 for fat. The remaining tissue was then processed and the paraffin sections were stained by a variety of methods which have been described previously.[7,9] Autoradiography was carried out on paraffin sections using Kodak NTB2 liquid emulsion and an exposure time of 28-35 days. Small cubes of each hepatic sample were used for electron microscopy. Initial fixation was glutaraldehyde was followed by osmic acid. After embedding in 'Epon', ultrathin sections were cut, stained with lead citrate, and examined [sic] in a Philips 300 electron microscope. The size of the hepatocytes was determined by tracing out large numbers of midzonal liver cells on standard-thickness paper, cutting out the silhouettes, and weighing them. The weights were referred to as size units. We have shown this to be an accurate method for comparing cell sizes by confirmatory planimetry and by studies of unicellular organisms, the size of which could be directly determined. Midzonal hepatocytes were also used for measuring on electron micrographs the length of rough endoplasmic reticulum per area of cytoplasm by Loud's method.[12]

Results

Controls and placebo infusions.

Four days after portacaval shunt, the liver cell size units in both the left and right lobes of the animals of group 1 decreased by about half (table I). In addition to the hepatocyte shrinkage, light-microscopic examination showed that the hepatocytes were irregular in shape and were depleted of glycogen. The lobules were smaller than normal and the reticulin framework was condensed around the central veins. The Kupffer cells were enlarged and filled with material which included ceroid and lipofuscin and in three animals hæmosiderin. A little fat was visible in the hepatocytes in 5 of 11 dogs. The number of hepatocyte mitoses was greater than normal.

The ultrastructural changes caused by Eck fistula within the four days were the same in the left and right lobes. The most prominent effects were decreases in the amount of rough endoplasmic reticulum with disruption and dialation of the cisternæ. Morphometric analysis showed that the area of rough endoplasmic reticulum per volume of cytoplasm was reduced to between half and a third the quantity found in the preoperative biopsy and the number of membrane-bound ribosomes was reduced. The quantity of smooth endoplasmic reticulum was increased. Some of the mitochondria were enlarged and showed distortion of their cristæ.

The autoradiographic studies gave quantitative confirmation of the impression of an increased mitosis-rate (table II). The number of hepatocytes undergoing D.N.A. synthesis in both left and right liver lobes was nearly 5 per 1000 (table II). The rate in 17 normal dogs including 6 that were subjected to sham dissections of the portal tract was recently established in our laboratories as 1.6±0.4 (S.D.) per 1000 cells in the left lobes and 1.6±0.5 (S.D.) in the right lobes.[9]

All the foregoing findings were almost precisely the same in the dogs of group 2 whose left lobes were infused with a heparin saline infusion for four days after Eck fistula (tables I and II).

Insulin infusion.

The infusion of 0.43 units per kg/day of commercial insulin into the left portal vein for four days after Eck fistula in group-3 dogs markedly reduced the hepatocyte shrinkage in the left lobes without affecting at all the acute atrophy of the right lobar hepatocytes. The amount of glycogen in th cytoplasm of the hepatocytes in the left lobe was still less than normal, but much more than in the atrophic right lobar hepatocytes. The Kupffer cells in both lobes were enlarged and full of ceroid and lipofuscin.

Ultrastructurally, comparisons of the left and right lobar hepatocytes showed that the liver cells exposed to insulin infusion were almost normal. The area of rough endoplasmic reticulum per volume of cytoplasm and the number of membrane-bound ribosomes were only slightly reduced; dilatation and disruption of the cisternæ were minimal. Glycogen granules were frequent, but the number of small fat vacuoles was increased. The changes in the hepatocytes in the right lobe did not differ from those seen in the controls.

Autoradiography showed that in the insulin-infused left lobes the number of cells undergoing D.N.A. synthesis had nearly trebled whereas

TABLE II—CELL DIVISION BY AUTORADIOGRAPHY IN THE LIVERS OF 63 DOGS

Group no.	No. of experiments	No. of labelled hepatocytes per 1000 hepatocytes (mean±S.D.)		P values*
		Left†	Right†	L4 *vs* R4
1	8	4·9±1·0	4·7±0·9	N.S.
2	6	4·6±0·8	4·7±0·9	N.S.
3	9	13·0±3·9	4·6±0·9	<0·001
4	4	15·6±2·0	5·3±1·0	<0·001
5	8	14·4±1·1	4·8±1·0	<0·001
6	8	4·9±0·9	4·3±0·6	<0·05
7	6	4·2±1·5	4·3±1·1	N.S.
8	7	11·8±1·2	4·5±0·8	<0·001
9	7	14·8±1·0	4·5±0·9	<0·001

*Comparisons were by Student's *t* test.
†In 17 unaltered or sham-operated dogs the left and right lobar values respectively were 1·6±0·4 and 1·6±0·5. The increase caused by Eck fistula is significant ($P<0.001$).

TABLE III—MORNING BLOOD-SUGARS DURING FOUR-DAY EXPERIMENTAL PERIOD

Group no.	No. of determinations	Blood-sugar (mg/dl) (mean ± S.D.)
Normal dogs	10	61 ± 9
1 and 2	20	63 ± 8
3	38	65 ± 13
4	13	71 ± 8
5	21	71 ± 11
6	24	65 ± 12
7	12	65 ± 10
8	24	63 ± 9
9	24	68 ± 13

D.N.A.-synthesizing cells in the right lobes of the group-3 dogs were not different in numbers from those in the untreated Eck fistula animals of groups 1 and 2.

The results in all the end-points measured were almost exactly the same in the dogs of group 5 when purified insulin was used instead of the commercial insulin (tables I and II). A pronounced but slightly reduced protective effect of the left lobes was still evident even when the insulin dose was reduced in group 4 to about a third of that used in groups 3 and 5 (tables I and II).

In all the groups morning blood-sugars on frequent occasions did not deviate significantly from normals in our laboratory (table III).

Glucagon infusion.

The administration of small (group 6) and large (group 7) doses of glucagon into the left portal vein for four days did not appreciably influence any of the changes caused by Eck fistula (tables I and II).

Insulin/glucagon infusion.

When glucagon was added to proven effective doses of insulin to make an isulin/glucagon molar ratio of 2/1 (group 8), the effect was no different than with insulin alone (tables I and II).

The animals of group 9 receive 100 times as much glucagon. Now the protection from hepatocyte atrophy in the left lobes afforded by insulin seemed partly lost. By one-way analysis of variance using Duncan's multiple range test with 95% confidence limits, the L4 hepatocytes were not in the same subset as the protected groups 3, 5, and 8, but neither did they fit with the control and pure glucagon groups 1, 2, 6, and 7. The badly affected left lobar liver cells were still significantly less shrunken than those on the right (table I). The autoradiographic observations with D.N.A. incorporation showed the same threefold increase in the left lobes as with insulin alone or as with 2/1 insulin/glucagon ratio (table II).

The light and electron-microscopic analysis did not show why the insulin protection had been partly lost in group 9. The amount of glycogen in the left lobar hepatocytes seemed almost normal, but the volume of rough endoplasmic reticulum was less than in the hepatocytes in the left lobes of dogs infused with insulin alone or insulin combined with lower doses of glucagon. Some mitochondrial abnormalities were also present.

Discussion

Eck thought[13] that a completely diverting portacaval shunt (Eck's fistula) in dogs was compatible with prolonged good health. This conclusion was refuted in 1893 by Hahn, Massen, Rencki[sic], and Pawlow[14] whose dogs with Eck fistula developed anorexia, weight loss, liver atrophy, and hepatic encephalopathy. The inability to explain these consequences caused Bollman[15] to write in 1961" "In the 83 years since it was first reported, the Eck fistula has been reasonably successful in hiding its secrets as well as in giving rise to many additional questions fundamental to an understanding of the functions of the intestine, liver, and brain." By then it had been widely accepted that the Eck fistuia syndrome was caused by a suboptimal volume as opposed to quality of hepatic blood-flow. This conclusion had apparent support from the fact that the portoprival complications could be minimised if the central tied off portal vein was revascularized with systemic venous blood (Child's portacaval transposition[3]) or with arterial blood.

The flow concept was undermined by a series of investigations in which two canine livers or fragments of the same liver were given different kinds of portal inflow.[7,9,16-20] The hepatocytes perfused with total portal flow or its pancreaticogastroduodenosplenic fraction became glycogen-rich, hypertrophic, and more active in cell renewal than the hepatocytes in the disadvantaged liver tissue which developed the histopathological features caused by Eck fistula despite apparently adequate replacement flow. Collectively, the enriching constituents thus demonstrated to be in portal blood were called hepatotrophic substances. It became obvious from biochemical[7,20][sic] and pancreatectomy or allo an-diabetes studies[8,9,20][sic] that insulin was the most important of these substances and that the well-known efficiency of insulin's removal during a first pass through hepatic tissue[21-23] made it relatively unavailable for a second liver or liver fragment. At the same time the benefit after portal diversion from flow augmentation procedures such as Child's portacaval transposition was explained. If insulin and other hepatotrophic substances were bypassed around a single liver, they would be returned to it in diluted form in direct relation to the total hepatic blood-flow.

The natural deduction from our earlier studies was that many of the secrets of the Eck fistula were manifestations of an altered hormone environment in which the most important change was deprivation of the liver of direct access to endogenous insulin. The experiments of a recent preliminary communication[10] and of this more complete report directly tested that hypothesis and with unequivocal results.

Non-hypoglycæmic insulin infusions for four days into the tied-off left portal vein after Eck fistula greatly reduced the left lobar hepatocyte atrophy, permitted the ultrastructure of the protected liver cells to remain essentially normal, and caused a threefold increase in the number of left lobar hepatocytes undergoing mitosis. There was no spillover effect in the right lobes. Glucagon by itself did not cause any of these changes, and it did not potentiate them when added to insulin at a 2/1 molar insulin/glucagon ratio. At a dose 100 times greater, glucagon may actually have reduced the benefit from insulin.

Thus it has been established that "hepatic insulinopenia" is the most important element in the liver injury of Eck fistula. It is ironic that this answer was so close to detection all the time. Hahn et al[14] definitely emphasised more than 80 years ago that, unless the portal vein was ligated above the last portal branch during the performance of Eck fistula, their dogs remained quite normal. This highest vessel drains the pancreas, but of course the endocrinological significance of that fact was not then known. Because of that same ignorance of hormones, speculation by Rous and Larimore[24] was vague about the possibility as they saw it in 1920 that portal blood might have special liver-supporting constituents. A perplexing observation of our own from almost 20 years ago contained a strong clue.[25] It was noticed that dogs with Eck fistula plus insulin-treated alloxan diabetes remained in much better health than animals with Eck fistula alone in that the diabetic dogs gained weight and were spared all evidence of encephalopathy. The now-obvious explanation is that peripheral insulin concentrations of two or three times normal are required in diabetics to maintain normoglycæmia when insulin is given systemically,[26] thus inadvertently providing a compensatory hepatic arterial increase in the hormone. Conversely, in untreated alloxan-diabetic rats with unaltered hepatic circulation, Reaven, Peterson, and Reaven[27] have demonstrated acute ultrastructural changes in the liver cells that were remarkably like those caused by Eck fistula.

In the past we have emphasized[7,10,20] that multiple factors including collaborating hormones as well as nutrients undoubtedly contribute to the total hepatotrophic effects of portal blood. It would be regrettable now if the very clarity with which insulin has emerged as a principal portal hepatotrophic substance were to obscure the search for contributory factors. The observation that the insulin protection in our experiments was not quite complete may be a reflection of missing ancillary substances although this could also be explained by the inability of infusion pumps to make appropriate physiological modulations of delivery.

However, it is particularly in the area of cell growth control and regeneration that efforts must not be made to use insulin as a monolithic explanation for everything.[9] This can be made clear with some observations about hepatocyte renewal under differing portoprival conditions. In livers of Eck fistula dogs of the present study, in rat livers after portacaval shunt,[28] and in the portoprival hepatic fragments of double liver models[8,9] the atrophic and insulin-starved hepatocytes have been demonstrated by autoradiography to have mitosis-rates that paradoxically were three or four times normal. The stimulus for the low-grade hyperplasia is unknown, but it is presumably a response to an increased hepatocyte death-rate. The

provision of exogenous insulin in the present studies or of endogenous insulin in the complicated double liver fragment experiments[8,9] provoked a sustained burst of proliferation beyond the already heightened mitotic background.

These results were consistent with those in the important study by Younger, King, and Steiner[29] who allowed rats to be alloxan-diabetic for a month before treating them with insulin. Although the livers were thought to already contain a higher than normal number of hepatocytes, the proliferative response to insulin was spectacular, being similar to that after a 68% liver resection. However, Younger and his colleagues[29] also showed that diabetic rats retained a potent although subnormal ability to regenerate their livers after an actual hepatic resection even if insulin treatment was withheld. Accordingly, it is unlikely that any single control factor will be the sole explanation of regeneration. Holley[30] and Leffert[31] have summarised the dozens of substances, hormonal (including insulin) and others, that can initiate and regulate cell growth in tissue-culture systems. There is no reason to doubt the relevance of their comments concerning the complexities of growth control to in-vivo situations.

Even so, the prospect seems promising of favourably influencing regeneration and recovery after acute liver injury in laboratory animals and ultimately in man by the simple expedient of intraportal insulin therapy. Earlier,[8,10] [sic] we speculated that insulin for this purpose might have an augmented benefit if combined with glucagon. A recent study on regeneration by Bucher and Swaffield[32] has provided support for such combination therapy. However, the failure in our studies reported herein, as well as in past equally well-controlled ones, [8,9,20] [sic] to identify a beneficial additive role of glucagon, coupled with the slight possibility that in high doses it might have even cancelled some of the insulin benefit, would cause us either to omit glucagon or to use it in small amounts only.

As such possibilities are considered, the relevance of canine portal physiology to man will be raised. It has been pointed out elsewhere[1] that the same general light and electron microscopic changes have now been seen after portal diversion in the livers of rats, dogs, baboons, and humans with some variation in degree. Thus, the hepatic injury of Eck fistula is common to all species so far studied. Fortunately, the most serious metabolic consequences have seemed to selectively spare rats and man, a species difference that has made it feasible to perform the procedure with benefit to patients with glycogen-storage disease[33] and homozygous type-II hyperlipidæmia.[34] These applications accept a trade-off of distinctly suboptimal conditions of liver perfusion in return for metabolic improvements that almost certainly derive from the suboptimal conditions.[20,33,34] [sic] A similar weighing of gains and losses is necessary for the traditional indications for shunting operations in patients with œsophageal varices who retain hepatopetal portal flow. An obvious argument can be mounted for a Warren-type operation which preserves much of the residual splanchnic flow to the liver including that returning from the pancreas.[35]

Appreciation that there is a broader interplay between the liver and pancreas than the classical metabolic ones of Madison[36] and Felig and Wahren[37] should have ramifications in understanding the complications of diabetes mellitus, the pathogenesis of liver disease, and numerous other clinical problems as has been pointed out by others.[38,39]

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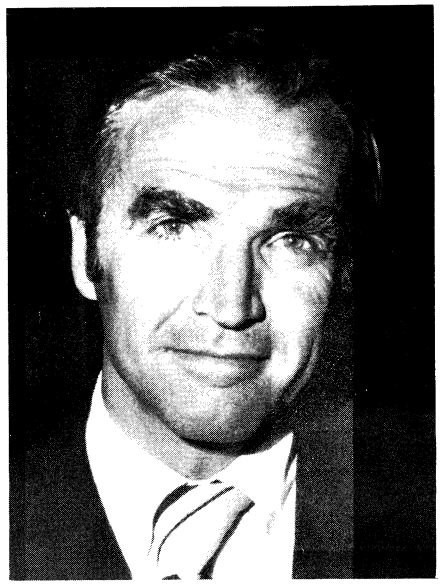
Karel B. Absolon



Richard Lillehei (1928-1981)

Dr. Karel Absolon was a young surgeon at the University of Minnesota. He had a profound interest in transplantation and hepatic surgery. His description of the first attempt at auxiliary liver transplantation was given at a conference in Lyon, France in May, 1965. Absolon, also a student of medical history and the author of a biography of Billroth, is in private practice in Washingtion, D.C.

The senior faculty member was Richard Lillehei, one of transplantation surgery's great pioneers and the first to attempt clinical pancreas and intestinal transplantation. Lillehei died on 1 April, 1981 while training for the Boston Marathon. He was 53 years old.

Experimental and clinical heterotopic liver homotransplantation

Revue Internationale d'Hepatologie, 15: 1481-90, 1965

Karel B. Absolon, Patrick F. Hagihara, Ward O. Griffen, Jr. and Richard C. Lillehei

Direct therapy of malignant tumors of the liver would necessitate excision of the organ and its replacement.[1,2] In benign diseases of the liver, such as cirrhosis in adults and biliary atresia in infants, which eventually results in hepatic failure, heterotopic liver implantation while the recipient liver is left intact may be the approach of choice. The first successful whole organ heterotopic canine liver homograft procedure was performed by Welch and his associates[3,4] and inspired a heterotopic liver homograft operation developed by the authors.[5]

The procedure previously tested on dogs consisted of placing the liver in the left side of the abdomen after splenectomy to gain space. Arterial inflow to the homograft was accomplished by anastomosing the hepatic artery to the aorta or its branches and venous outflow from the homograft by anastomosing the inferior vena cava of the graft to the inferior vena cava of the recipient. In dogs the graft portal vein was either tied or anastomosed to the portal vein of the recipient. In order to decompress the transplant, in case of proplonged ischemia which led to high resistance to blood inflow the portal vein graft was anastomosed to the graft inferior vena cava. The common duct was drained of its bile externally for functional evaluation. Azathioprine and Predisolone administration produced prolongation of graft function. In dogs with their own bile duct obstructed, these drugs prolonged bilirubin clearance by the graft. While some grafts under such therapy showed the typical mononuclear infiltration characteristic of rejection, other [grafts] remained histologically normal.[5]

Case Report

Preoperative status.

T.K.: A 13-month old infant was the product of a non-complicated spontaneous vertex delivery. He did well at home until four weeks when he developed icterus associated with dark urine and clay colored stools. At the age of 8 weeks an exploratory laparotomy was performed at an outside hospital facility which showed no evidence of extrahepatic bile ducts. The postoperative course was uneventful and the condition of the patient remained unchanged. At the age of 5 months he was admitted to the University of Minnesota Hospital for re-evaluation.

The physical examination revealed a jaundiced male infant in moderate respiratory distress and a distinct hepatic fetor, a distended, tense, fluid-containing abdomen and hepatosplenomegaly. Surgical re-exploration revealed a partial bowel malrotation and a rudimentary gall bladder containing clear mucoid material with no evidence of extrahepatic ducts.

A biopsy of the liver revealed distorsion of normal architecture with enlarged portal spaces and confluent bands of fibrous tissue (Fig. 1A). The liver parenchyma was divided into irregular nodules. The hepatic cell cytoplasm as well as the intra-lobular bile ducts contained abundant bile pigment. Within fibrous bands were numerous proliferating, newly formed bile ducts.

The laboratory findings revealed a urinalysis with 1+ albumin and qualitatively positive bilirubin. The hemoglobin was 10.5 gm.%, leukocytes were 11,600 per cu. mm. with 41% neutrophils and 51% lymphocytes. The prothrombin time was 13.2 seconds with a control of 11.4 seconds. A blood sugar of 76 mg.% and a total protein of 6.9 gm% with albumin of 3.4 gm.% and globulin of 3.5 gm.% was measured.

At the age of 11 months the patient was re-admitted for liver transplantation. The child had been maintaind on bile salts, 0.2 gm. three times a day and vitamins. In the interim, this lethargic, anorectic infant rapidly deteriorated after reaching a maximum weight of 16 lbs. and showed increasing jaundice. He had suffered multiple upper respiratory infections, otitis, bronchitis and pneumonia. The physical examination revealed a grossly retarded infant with no evidence of teeth, unable to roll, sit or vocalize. It appeared dyspneic and othopneic with yellow pale skin and decreased turgor. The respiratory rate was increased, the chest excursions shallow and grunting. The liver, which at the age of 5 months was palpable 5 cm. below the right costal margin, now was felt some 5 cm. lower; the liver edge was firm and sharp. The spleen was felt 6 cm. below the costal margin, approximately the same level as at the age of 5 months. The chest x-rays revealed changes in the right middle lung field compatible with bronchopneumonia.

The laboratory findings showed in the period six months prior.to admission (Fig. 4) fluctuation of serum alkaline phosphatase levels from 75 to 220 King Armstrong (K.A.) units per cent with an average of 152.4

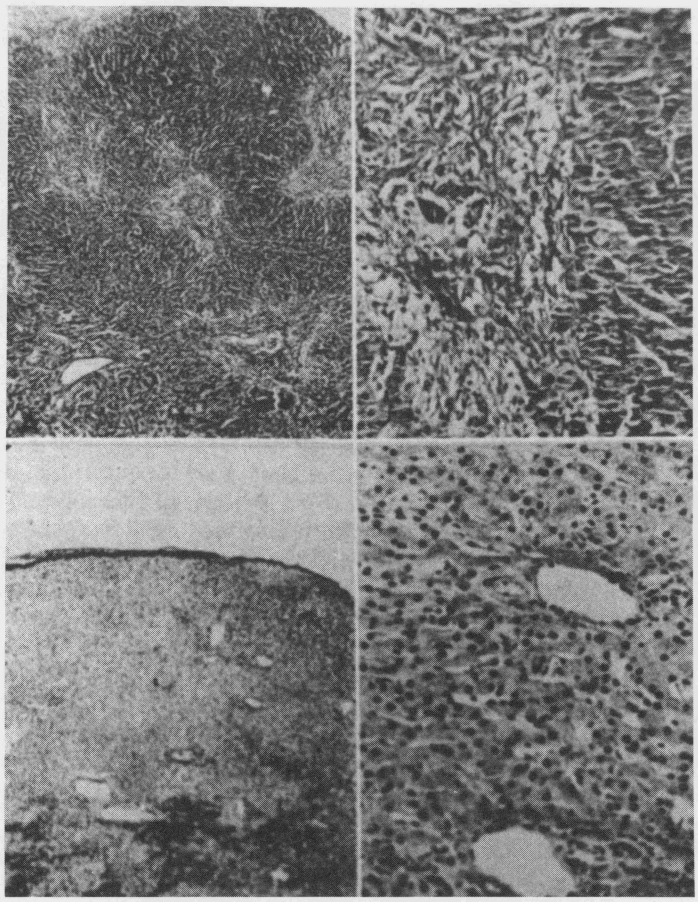


Fig. 1.— A. Upper: Patient's own liver characteristic of extra hepatic biliary atresia.
B. Lower: Liver homograft, 13 days after implantation.

K.A. units per cent. The total serum protein at the age of 5 months was 6.9 gm.%. (A/G: 3.4/3.5 gm.%) and now had changed to 6.2 gm.% (A/G: 2.7/3.5 gm%). The serum bilirubin varied from 8 to 9 units, the cephalin cholesterol was 2+ and the total cholesterol was 314 to 273 mg.%. The serum glutamic oxaloacetic transaminase varied from 124 to 274 pyruvic units per ml. (P.U.)(Av. 199.8 P.U./ml.) The serum glutamic pyruvic transaminase was determined on two occasions and was measured at 301 and 497 P.U./ml. Sputum cultures showed a varied flora (beta-hemolytic Streptococci, Pseudomonas Aeruginosa and Klebsiella organisms). After admission for consideration for transplantation, this operation had to be deferred several times because of the patient's serious condition punctuated by recurrent episodes of semi-coma, pneumonitis, and partial intestinal obstruction. The patient's course was characterized by periods of electrolyte imbalance such as hyponatremia, perhaps conditioned by administration of diuretics and Aldactone and moderate anemia and leukocytosis which represented his response to various infections. Progressive jaundice associated with anemia necessitated support with blood and human serum albumin transfusions. The bilirubin during the last month prior to transplanation varied from 11.5 to 36 mg.% total (av. 21 mg.%) and 5 to 20 mg.% one minute (av. 10.7 mg.%). The prothrombin time on several occasions was slightly elevated, reaching the highest level of 15.2 seconds with a control of 10.8 seconds.

Operation:

On November 3, 1964 a heterotopic liver graft procedure was performed. The liver was removed from a 2 1/2 year old patient suffering from complete transposition of the great vessels and dextrocardia with situs inversus. The recipient weighed 7 kg. and the blood group was O Rh- (C-, D+, hr+); the female donor weighed 9 kg. and had an A+, Rh+ (C, D, E, hr+) blood group.

Preparation of donor: The donor was placed on complete cardiac bypass utilizing the DeWall bubble oxygenator and a Mustard operation was performed for corrective repair of transposition of the great vessels under moderate hypothermia (Rectal T. 33° C. and esophageal 19-25° C.) The perfusion rate was 500-810 ml. of blood per minute for 109 minutes. Four attempts at discontinuing the bypass were unsuccessful and the heart did not resume blood pressure sustaining action despite vigorous measures. While under total body perfusion the legal permit to remove the liver for grafting was obtained. Five hundred ml. of 0.9% salt solution and 150 ml. of 5% dextrose in water and 500 ml. whole blood were added to the perfusate as well as antibiotics, 25 mg. Chlorpromazine and 18 mg. Dibenzyline. The perfusion was continued under deep hypothermia (10-13° C at 180-300 ml./min. flow rate) for 4 hours until the recipient was ready to receive the graft.

A midline incision from the xiphoid process to the pubis in addition to the bilateral transverse sternal thoracotomy used for the unsuccessful cardiac operation gave good exposure. The liver was displaced to the left of the normal position, as is typical of a situs inversus. As the venous return from the lower half of the body was through the femoral vein, the thoracic portion of the vena cava could be ligated soon [sic] after freeing the triangular ligaments of the liver. Easy access to the common hepatic artery was obtained after transection of the esophagus and the gastrohepatic ligament. The vessels of the celiac axis were dissected, the splenic and left gastric arteries divided. The portal vein and infradiaphragmatic vena cava were dissected and an end-to-side portacaval shunt established. At this point the recipient was ready to receive the homograft. Blood was drained from the

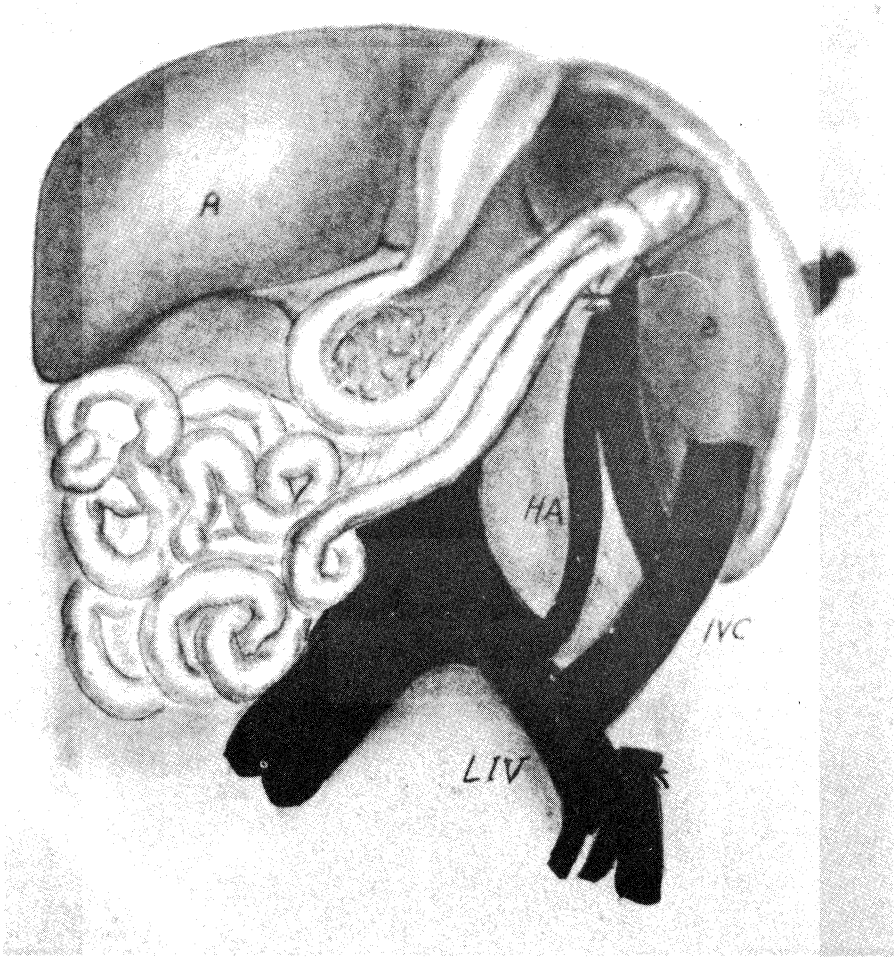


Fig. 2.— Heterotopic liver homotransplantation. A - Recipient liver. - B - Donor liver. - IVC - Donor Inferior Vena Cava. HA - Donor Hepatic Artery. - LIV - Racient Left Iliac Vein.

perfusion system and replaced with a chilled solution of 500 ml. 5% dextrose in water and 5% low molecular weight Dextran. After removal of the graft in vitro perfusion was started through the hepatic artery and portal vein for 8 minutes with a chilled solution of Tis-u-Sol, 5% low molecular weight Dextran and 5% dextrose in water buffered with bicarbonate and Tris to a pH of 7.4. All blood and the anticoagulant were thus flushed from the organ.

Preparation of the recipent and liver transplantation: (Fig. 2, 3) Through an oblique abdominal incision, the peritoneal cavity was entered after freeing the left colon, ligating the inferior mesenteric artery and isolating the aorta, vena cava and iliac vessels; a splenectomy was performed. Since more space was needed, a left nephrectomy was [sic] done. Interestingly, the left iliac vein was of larger caliber than the vena cava. The vena cava of the graft serving as its venous outflow (opposite to the normal flow) was therefore anastomosed end-to-side to the iliac vein. The hepatic artery with a cuff of aorta was anastomosed end-to-end to the iliac artery (Fig. 2). Normal blood perfusion was established after 37 minutes of ischemia which included 8 minutes of washing out the organ. Since the graft common duct was unusually small, a cholecystojejunostomy just beyond the ligament of Treitz was done to provide the outflow tract from the graft. The total blood [sic] loss was 50 ml. and the patient tolerated the procedure well.

Postoperative course: (Fig. 4) For immunosuppression the patient was placed on Azathioprine 4 mg./kg/day and prophylactic antibiotics. In addition he received 25 mg. Prednisolone on the day of operation and 25 mg. Cortisone. For the next two days, 8 mg. of Cortisone was given and replaced subsequently with 7.5 mg. Prednisolone daily in 3 divided doses. The patient received 50 R tissue dose of cobalt ionizing radiation to the area of the liver homograft.

The patient started having stools of normal color and infant character on the second postoperative day at which time some oral administration was initiated. The serum bilirubin decreased to 2.1 mg.%/min. and 4.1 mg.% total on the 7th postoperative day. Serum glutamic oxalacetic and pyruvic transaminase levels elevated preoperatively, increased remarkably at first, but then regressed toward preoperative levels on the 4th postoperative day. (SGOT, SGPT- 203/483 P.U./ml.).

On the 9th postoperative day the infant developped [sic] signs of peritoneal irritation with ascites. The transaminase levels increased again (SGOT/SGPT-1655/1705 P.U./ml.) as well as the serum bilirubin (1min/total-5.3/8.5 mg.%). Drainage of bile accumulation from a sloughed stump of the homograft common duct resulted in a decrease of transaminase levels (SGOT/SGPT 83/332 P.U./ml.) and serum bilirubin (1 min/total 2.4/6.0 mg.%). Blood, peritoneal and spinal fluid cultures revealed Klebsiella infection to which the patient succumbed on the 13th postoperative day despite massive antibiotic therapy and continuing liver homograft function. On post mortem examination the liver was well preserved grossly and histologically (Fig. 1B). The vascular anastomoses were patent and angiograms of the vessels showed a normal pattern (Fig. 5).

Other laboratory findings: Three postoperative urinalyses showed traces of albumin and white blood cells; the urine bilirubin was positive twice and negative on two occasions. The white blood cell count varied from 13-37 thousand with predominant neutrophils. No coagulation defect

Fig. 3.— Heterotopic liver homotransplantation. - Note incision and position of heterotopic graft 1 week post operative.

was noted clinically. The platelets were counted at 39-364 thousand/cmm and the prothrombin time varied from normal to 63% normal. The partial thromboplastin time and a thromboelastogram were within normal limits. The erythrocyte sedimentation rate was slightly elevated. The alkaline phosphatase levels in the serum ranged from 25 to 115 K.A.U.% and the total proteins varied from 4.3 to 4.9 gm.% (A/G-2.3/1.8 to 2.7/2.2 gm.%). A moderate hyponatremia continued in the postoperative period.

Discussion.

Attempts at liver transplantation appear most justified in progressive liver failure due to noncancerous affections, if early death is predictable. Heterotopic homotransplantation appears to be the procedure of choice in cirrhosis or biliary atresia, as the recipients may not require total hepatectomy. It is a less traumatic and technically demanding operative procedure; vascualr anastomoses are accomplished while the host vessels are serially interrupted. Bypass and anticoagulation are not required as is the case with orthotopic liver homografts. The retention of the host's liver provides some hepatic function during the establishment of the homograft. A homograft may provide a temporary respite which may be lifesaving in case the recipient liver possesses any potential to recover.

The desirability [sic] of accomplishing liver homograft decompression by portacaval anastomosis must be weighed against the advantages of having portal flow through the homograft as may be accomplished by an anastomosis between the graft portal vein and the recipient portal system. Ischemia may not be as deleterious to the human liver as it is to the dog liver.[5] Further clinical inquiries are stimulated by the experimental results of Starzl et al in dogs who have shown life sustaining graft function of many months duration.[9]

The difficulty of obtaining liver grafts will necessarily hamper efforts in this area of clinical transplantation work. Suitable donors such as infants dying of cardiac abnormalities commonly have visceral anomalies making dissection technically difficult. In two other infants with biliary atresia in whom attempts at liver transplantation were made, the donor liver was unsuitable because of either size or gross changes produced by a prolonged period of ischemia.

Serum bilirubin levels are an inaccurate method of evaluating liver homograft function. Serum transaminase and alkaline phosphatase levels are nonspecific.[7] The general condition of the patient and the presence of bile in the stools are the simplest procedures. Bile bilirubin content and concentration are the most reliable laboratory procedures with which to follow homograft function.[5] External bile drainage and an internal anastomosis would appear the most practical solution in the management of a heterotopic liver homograft in patients with biliary atresia.

The immunosuppressive regimen with Azathioprine and steroids was a contributory factor in the fatal infectious complication in this infant. The desirability [sic] of exploring other methods of homograft tolerance production such as lymphoresis through the thoracic duct with the resulting development of lymphocytopenia and hypoglobulinemia is becoming quite evident.[7,8] Such mechanical agency in combination with immunosuppressive chemicals of a different nature or reduced dosage will become the approach of choice.

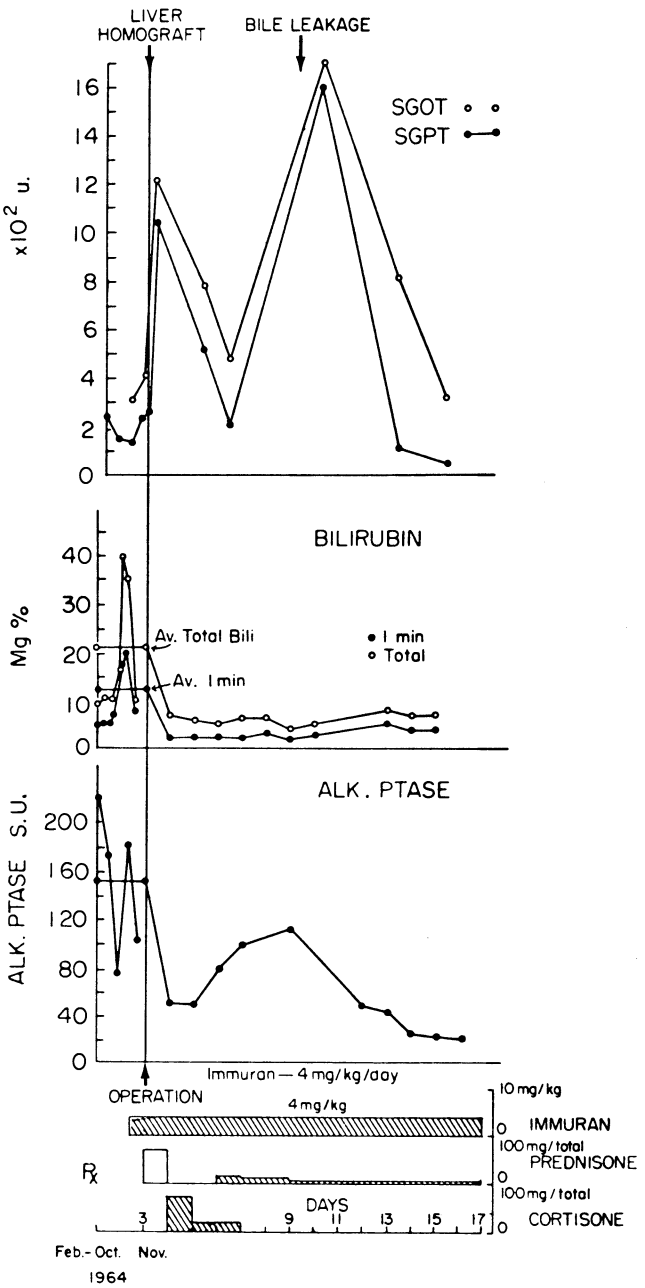


Fig. 4.— Heterotopic liver homotransplantation. Transaminase, serum bilirubin and alkalin phosphatase liver.

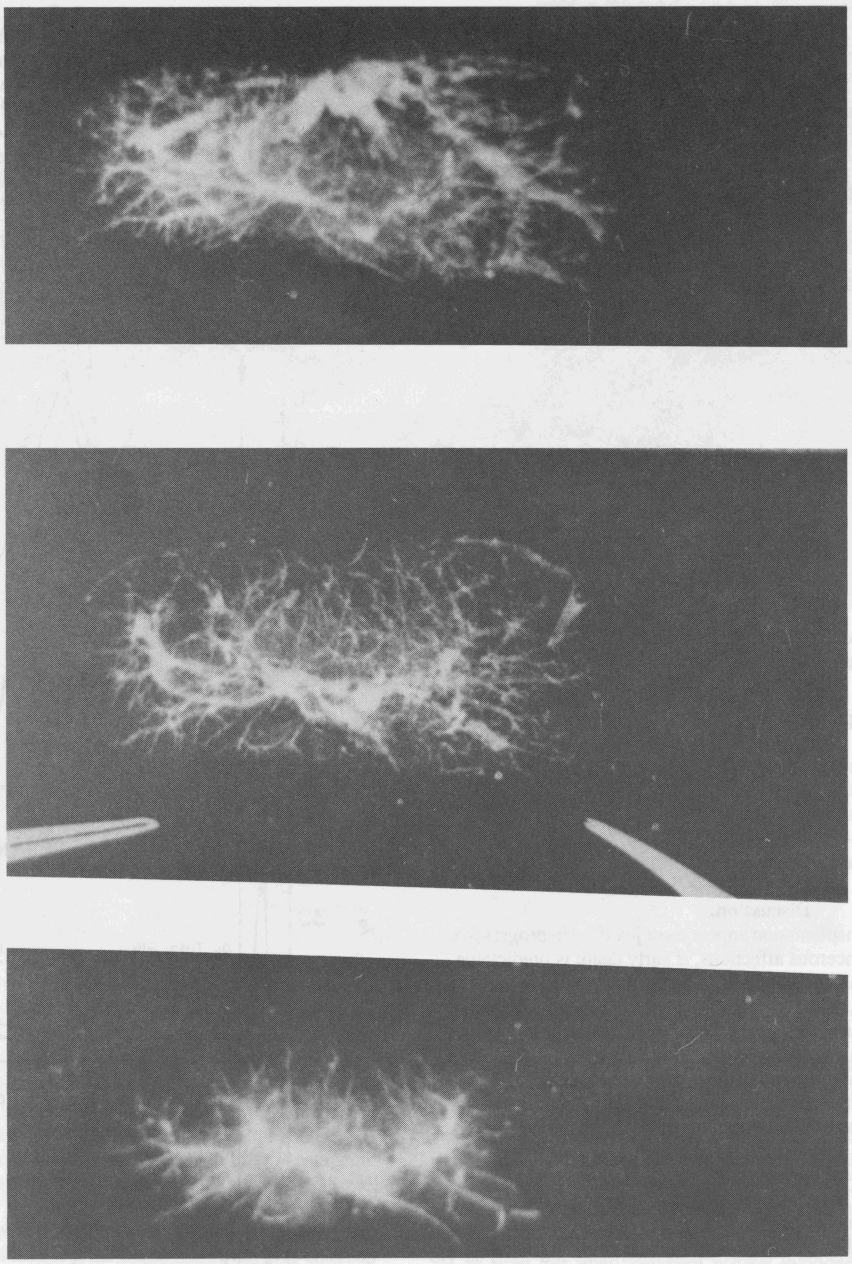


Fig. 5.— Postmortem angiogram of hepatic homograft. Upper: hepatic vein. - Middle: portal vein. - Lower: hepatic artery.

Summary.

The problems of heterotopic liver transplantation in the human were discussed and related to experimental studies in the dogs. A heterotopic liver homotransplant appears the optimal grafting method in the application of this type of experimental therapy to infants with biliary atresia.

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C. G. Halgrimson

Charlie Halgrimson was a resident in surgery at the University of Colorado when there was great interest in the potential use of auxiliary liver transplantation. His experimental studies showing that host portacaval shunt protects the liver graft and a description of several clinical trials of auxiliary transplantation were given at the Central Surgical Association in 1966. The practical problems and probable limited value of auxiliary liver transplantation were evident. Halgrimson subsequently served as Vice Chairman of the Department of Surgery from 1972 to 1980 and as Acting Chairman thereafter. He now is Associate Dean of the University of Colorado School of Medicine, Denver, Colorado.

Auxiliary liver transplantation: effect of host portacaval shunt

Archives of Surgery, 93: 107-18, 1966

Charles G. Halgrimson, Thomas L. Marchioro, Tanous D. Faris, Ken A. Porter, George N. Peters and Thomas E. Starzl

This report is concerned with a further evaluation of auxiliary canine homotransplantation of the liver and with the attempted application of such a procedure for the treatment of three patients.

Methods

There were 33 mongrel dogs weighing 17.7 to 22.2 kg (average, 17.5 kg) (39 to 49 lb [average, 38.5 lb]) used as recipients. The experiment was completed in 11 animals. The other 22 died early of anesthetic, technical, and septic complications and are excluded from further discussion. In the 11 test dogs the donors (weight 9.5 to 20 kg [21 to 44 lb]) were smaller than their recipients, the average weight being 13.6 kg (30 lb). The general surgical techniques and the studies obtained postoperatively were similar to those described in previous publications.[1,2] The average ischemia time for the cooled homografts was 44 minutes. Postoperatively, 2 to 8 mg/kg azathioprine were administered daily.

All animals received a completely diverting portacaval shunt. Immediately thereafter, the homotransplantation was performed. The animals are grouped into two categories.

Group 1 (Eight Animals).— Both of the inflowing vessels to the homograft were proved to be patent by premortem angiograms and by autopsy studies (Fig. 1, *A*).

Group 2 (Three Animals).— The inferior vena caval to portal venous anastomosis was shown to be occluded (Fig. 1, *B*). In these experiments, both the autologous liver and the homograft had only an arterial blood supply.

Results

General Observations.— There was no significant difference in the postoperative clinical course between the two groups of animals. Dogs of group 1 lost an average of 2.4 kg (5.3 lb) compared to 3.6 kg (7.9 lb) in group 2. All animals had transient rises in serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) with return to normal for varying periods before death or sacrifice; changes in alkaline phosphatase were similar but did not return to normal. Progressive anemia was invariably noted.

Group 1 Animals.— Terminally two of the eight animals became jaundiced, with maximum bilirubins of 5.1 and 8.2 mg/100 cc, respectively. The dogs lived for 24 to 150 days (average 58 days). Two were killed in apparent good health; two died of unknown causes; and four died of infection.

At autopsy the host livers appeared somewhat small but were otherwise normal. Similarly, some degree of gross atrophy affected the homografts. The weights of the homografts and the autologous livers (24 to 150 days after transplantation) are listed in Table 1. Marked homograft atrophy was present in only one animal. Since the donor animals were generally smaller than the recipients, these postmortem liver measurements were related to the total body weights of the respective animals at the time of transplantation. The weights of the homografts in relation to the original donor body weights averaged 2.03%. The mean ratio of autologous liver weights to the original recipient body weights was 1.79%. Since the predicted liver weights per total body weight ratio should be approxi-

TABLE 1.—*Dogs With Patent Portal Venous and Hepatic Arterial Inflow to the Homograft*

Dog No.	Survival, Days	Weight of Transplant, Gm	Weight of Host Liver, Gm
1	63	460	260
2	150	215	240
3	39	300	376
4	35	26	496
5	24	284	264
6	57	460	435
7	29	335	370
8	63	360	500
Average	58	305	368

Autopsy transplant liver weight per donor body weight: 2.03%.

Autopsy host liver weight per host body weight at time of operation: 1.79%.

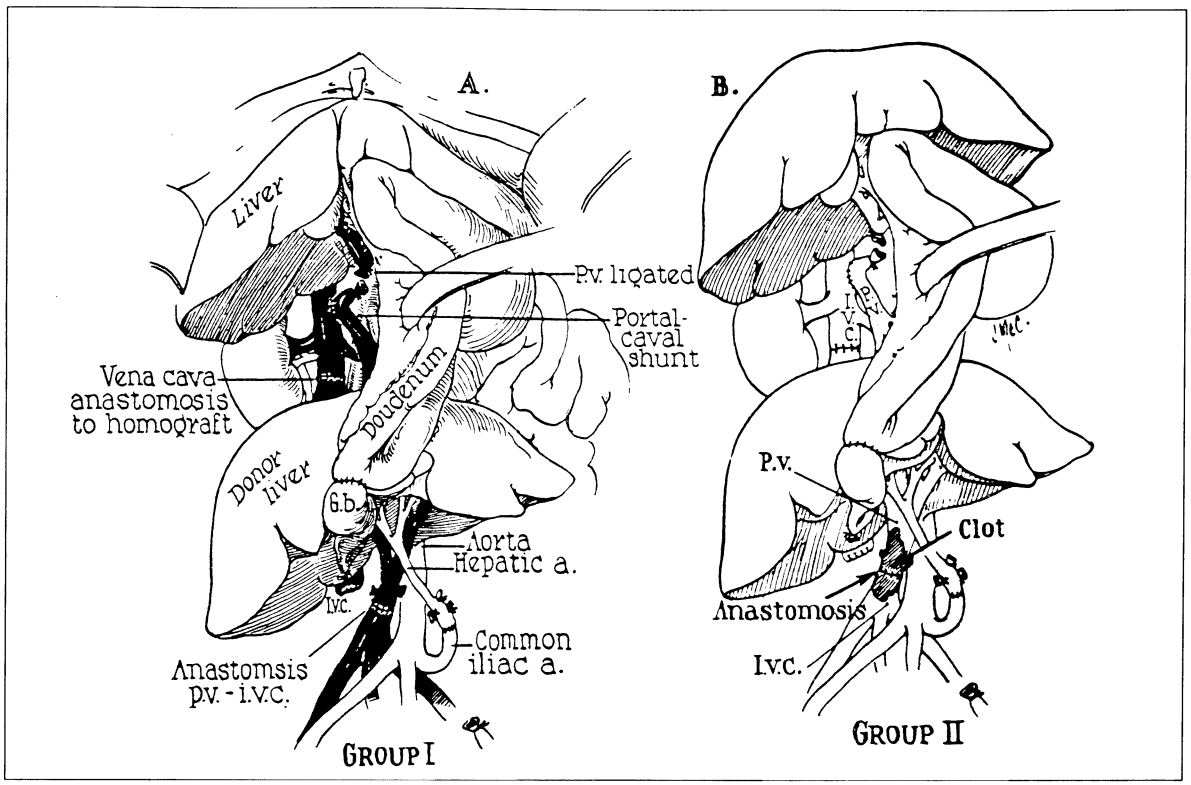

Fig. 1.— Experimental design of auxiliary canine homotransplantation of the liver, in conjunction with host portacaval shunt. *(A) Group I Series:* Note that the homograft receives a double blood supply, the portal inflow being of systemic venous origin. Except for the host portacaval shunt, the procedure is essentially that described by Welch[6] (B) Group 2 Series: In these dogs the portal venous anastomosis clotted. Thus, both the host liver and homograft received only an arterial blood supply.

mately 2.8%,[3,4] it seems likely that both organs underwent relative atrophy, the host liver slightly more than the homograft.

Histopathology of Group 1 Animals.— The eight host livers examined at autopsy, 24 to 150 days after operation, all showed atrophy of the centrilobular hepatocytes accompanied by collapse of the central part of the lobular reticulin framework (Fig. 2). Similar changes were present in the one biopsy that was obtained at 35 days. Seven of the eight livers contained moderate to large quantities of hemosiderin in the Kupffer cells. Centrilobular cholestasis with bile "thrombi" was present in four of the livers.

In six of the eight transplanted livers examined at autopsy there was absence of hepatocytes and complete collapse of the lobular reticulin. The liver cells were replaced by mesenchymal cells. Portal tracts and central canals were crowded together. Areas of hemorrhage and cellular infiltration, either mononuclear or polymorphonuclear, were frequent. Of the two less severely affected livers, one from a dog dying at 24 days (dog 5) showed necrosis of the centrilobular and midzonal hepatocytes together with collapse of the central part of the lobular reticulin framework, but no cellular infiltration or cholestasis. The other, from an animal killed at 63 days (dog 8), showed atrophy of the centrilobular hepatocytes, increased central and portal connective tissue, and bands of reticulin which linked portal tracts and cut across lobules producing pseudolobules. A small number of mononuclear cells were present in the portal tracts of this homograft, but only about 10% of these possessed pyroninophilic cytoplasm. There was cholestasis, some proliferation of bile ductules in the portal areas, and much hemosiderin in macrophages. Similar, though less severe, changes were present in this animal's homograft when it was biopsied at 35 days (Fig. 3).

Group 2 Animals.— None of the animals became jaundiced at any time; two were killed after 136 and 105 days and the third died of pneumonia after 73 days.

Two of the three host livers were small but were otherwise normal. The homograft livers showed a marked degree of atrophy but maintained normal gross architecture (Table 2). The weights of the homografts in relation to the original donor body weights averaged 0.72%, whereas the average weight ratio of autologous livers to the original recipient body weights was 1.52%. When compared to predicted weights, both sets of livers showed atrophy, affecting the transplant more than the host organ.

Histopathology of Group 2 Animals.— The three host livers examined at autopsy, 73 to 136 days after operation, all showed atrophy of hepatocytes, most prominent centrally but present to a lesser degree in the other zones of the lobules. This was accompanied by collapse of the central reticulin and accumulation of fat in the hepatocytes. Similar changes were present in the two biopsies that were examined at 35 days. Cholestasis was present in two livers.

In two of the three auxiliary transplants the hepatocytes had disappeared leaving only mesenchymal cells and the collapsed, condensed lobular framework of the liver. The third transplant, from a dog (1) killed at 136 days, showed increased reticulin and fibrous tissue in the portal areas and around the central veins. Connective tissue strands joined portal tracts and cut across lobules forming pseudolobules. Many mononuclear cells were present in the portal tracts. The cytoplasm of about 30% of these was pyronin-positive, and several of the cells were in mitosis. Macrophages in the portal areas contained large amounts of hemosiderin. Several of the hepatic arteries were narrowed by fibrous intimal thickening. The biopsy obtained from this liver at 35 days showed more cellular infiltration and less arterial damage (Fig. 4).

Report of Cases

Case 1.— A 50-year-old man was known to have alcoholic cirrhosis since 1957. In October 1964, he developed intermittent tarry stools and

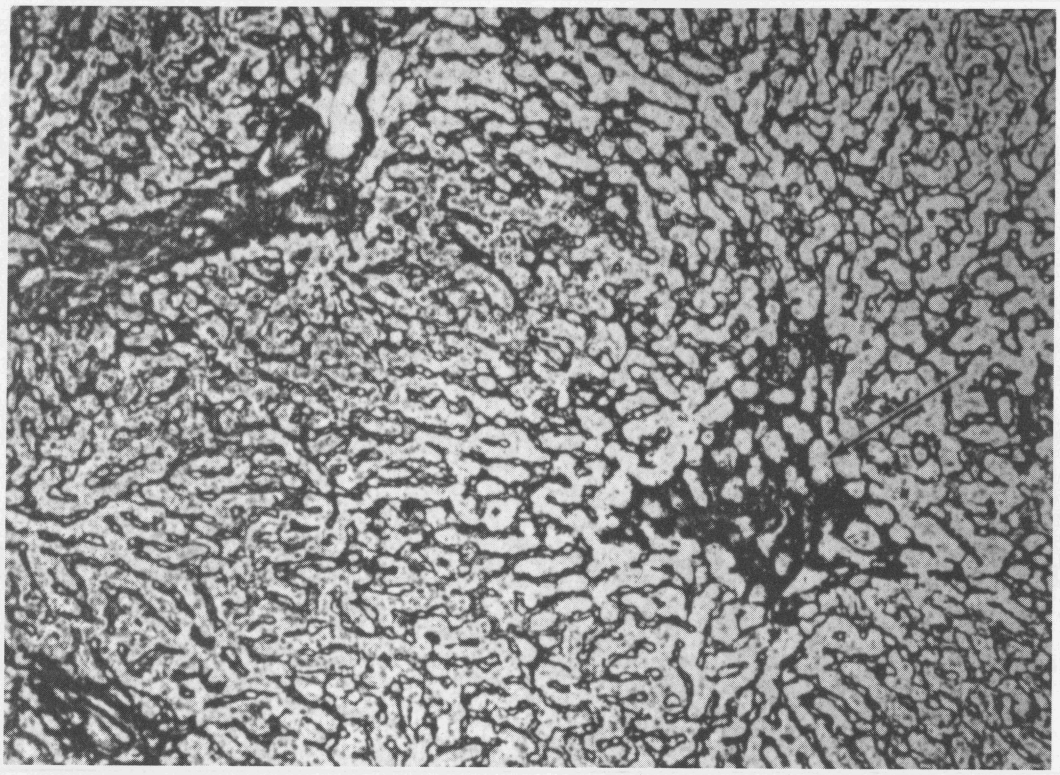

Fig. 2.— Own liver of dog 8 from group 1. Atrophy of the centrilobular hepatocytes has permitted collapse of the central part of the lobular reticulin framework (*arrow*) (reticulin stain, X 80).

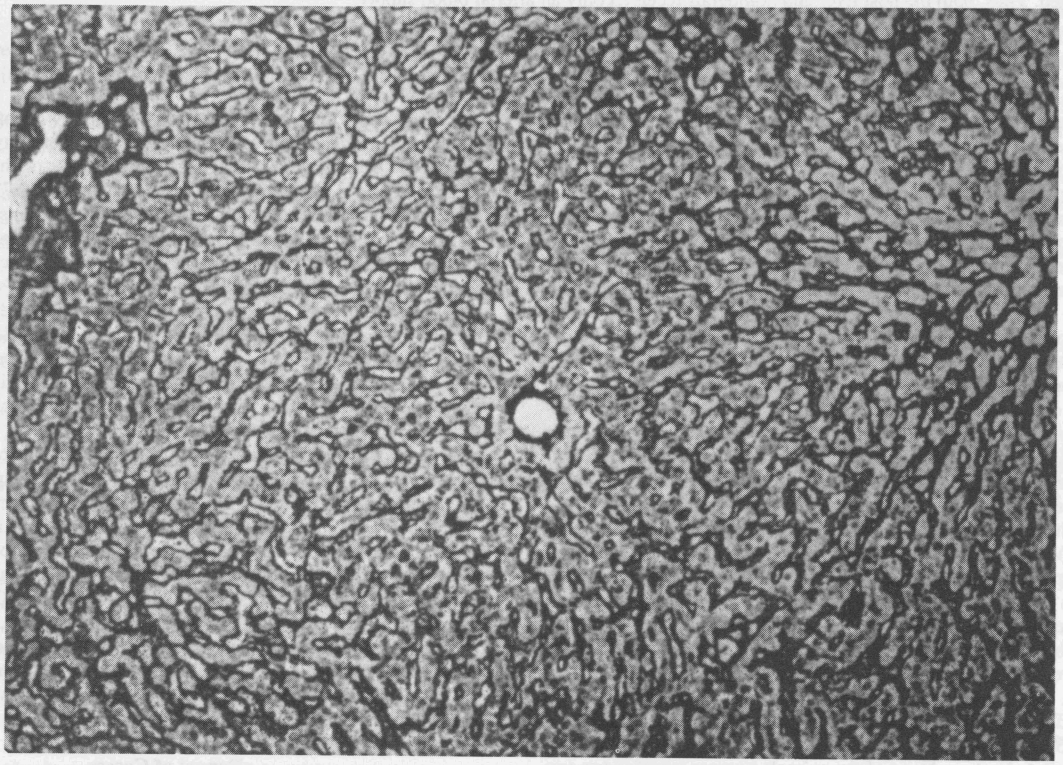

Fig. 3.— Biopsy at 35 days of auxiliary hepatic homograft from dog 8 (group 1). The lobular reticulin framework is normal. Twenty-eight days later this liver showed increased central and portal connective tissue and some pseudolobules (reticulin stain, X 80).

TABLE 2.—*Dogs With Patent Hepatic Arterial Supply Only*

Dog No.	Survival, Days	Weight of Transplant, Gm	Weight of Host Liver, Gm
1	136	82	232
2	73	36	150
3	105	150	410
Averages	105	89	264

Autopsy transplant liver weight per donor body weight: 0.72%.

Autopsy host liver weight per host body weight at time of operation: 1.52%.

jaundice. Physical examination after admission on Jan 26, 1965, revealed a tremulous, icteric, wasted man with an increased abdominal venous pattern, an enlarged tender liver, ascites, palmar erythema, bilateral varicose veins, and peripheral edema. Hematocrit reading was 42%; leukocyte count, 5,100 cu mm; total serum bilirubin, 22.5 mg/100 cc with 14.5 mg/100 cc direct; total serum protein, 6.6 gm/100 cc; albumin, 2.69 gm/100 cc; gamma-globulin, 1.68 gm/100 cc; alkaline phosphatase, 7.55 Bessey Lowry units; SGOT, 240 Sigma-Frankel units; prothrombin time, 61%; 4+ urine bile; blood urea nitrogen (BUN), 11 mg/100 cc; and fasting blood sugar, 90 mg/100 cc. There was progressive deterioration of liver function with increasing mental confusion; bilirubin rose to almost 40 mg/100 cc (Fig. 5). On the 22nd hospital day, he began to bleed massively from esophageal varices. An emergency end-to-side portacaval shunt was performed with prompt cessation of the hemorrhage. He became disoriented on the second postoperative day.

Three days after performance of the portacaval shunt, a cadaveric auxiliary hepatic homograft was placed in the right paravertebral gutter. Both donor and recipient were A positive blood type. The donor was a 79-year-old man who died after a cardiac arrest in the operating room. External and internal cardiac massage were carried out for 105 minutes before death was pronounced. Total body perfusion was then started with an electrolyte-primed extra-corporeal circuit into which a cooling coil was incorporated.[5] After 108 minutes of extracorporeal perfusion, the liver was removed and further perfused with intraportal cooled lactated Ringer's solution. Forty-seven minutes later the homograft was revascularized in the recipient, attaching the celiac axis to the side of the terminal aorta. Portal venous inflow was from the distal end of the transected terminal inferior vena cava. Hepatic venous outflow was provided by anastomosing the suprahepatic donor vena cava to the proximal end of the transected recipient vena cava. Roux-en-Y internal biliary drainage to the jejunum was established.

A tracheostomy was performed immediately after surgery because of severe respiratory distress. Postoperatively, there was an almost immediate clearing of his sensorium. Bilirubin and prothrombin times were strikingly improved (Fig. 5); other liver functions were not markedly altered. His immunosuppressive regimen is depicted in Fig. 5.

During most of the next three weeks, he remained lethargic but oriented. There were numerous postoperative complications including several bouts of gastrointestinal hemorrhage, urinary retention, reversible failure, electrolyte abnormalities, and purulent tracheobronchitis due to *Pseudomonas aeruginosa* and *Staphylococcus aureus*. In addition, homograft rejection was diagnosed and treated on the eighth postoperative day, after which there was slow deterioration of function. Progressive leukopenia was observed from the 11th day onward. Death occurred on the 22nd day after transplantation.

At autopsy, the immediate cause of death was thought to be necrotizing bronchopneumonia with multiple pulmonary abscesses. The

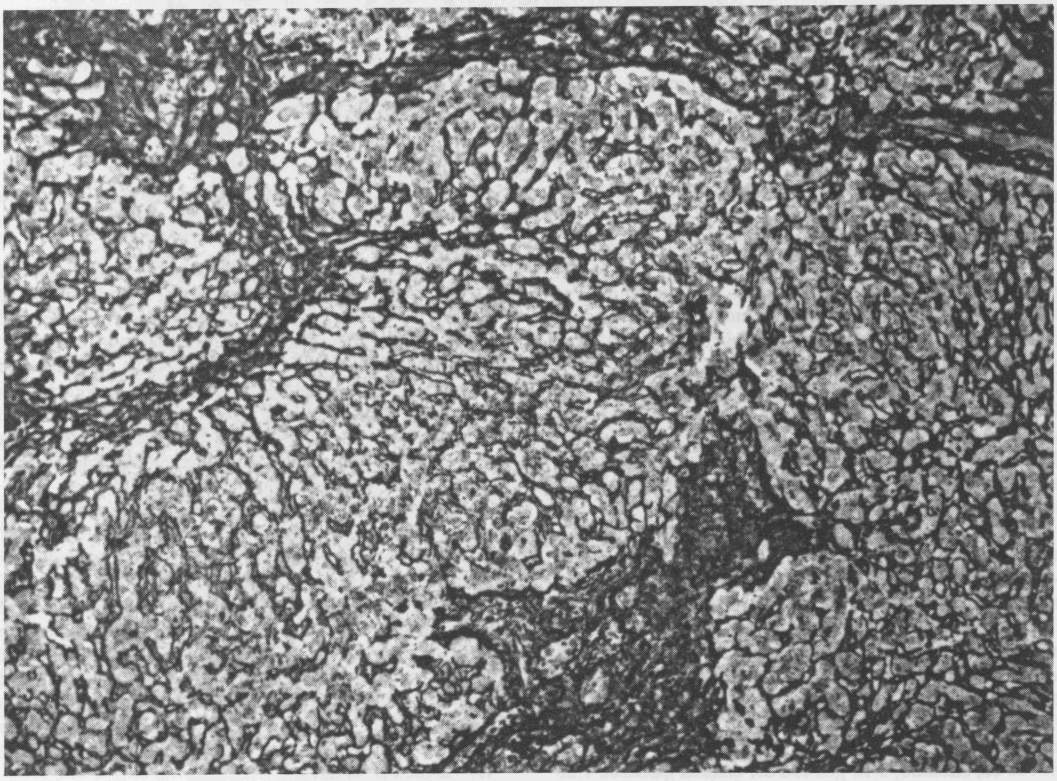

Fig. 4.— Biopsy at 35 days of auxiliary hepatic homograft from dog 1 (group 2). The normal lobular architecture has been disrupted by bands of connective tissue which link portal tracts and some central veins. There is increased reticulin in the portal areas and around the central veins (reticulin stain, X 80).

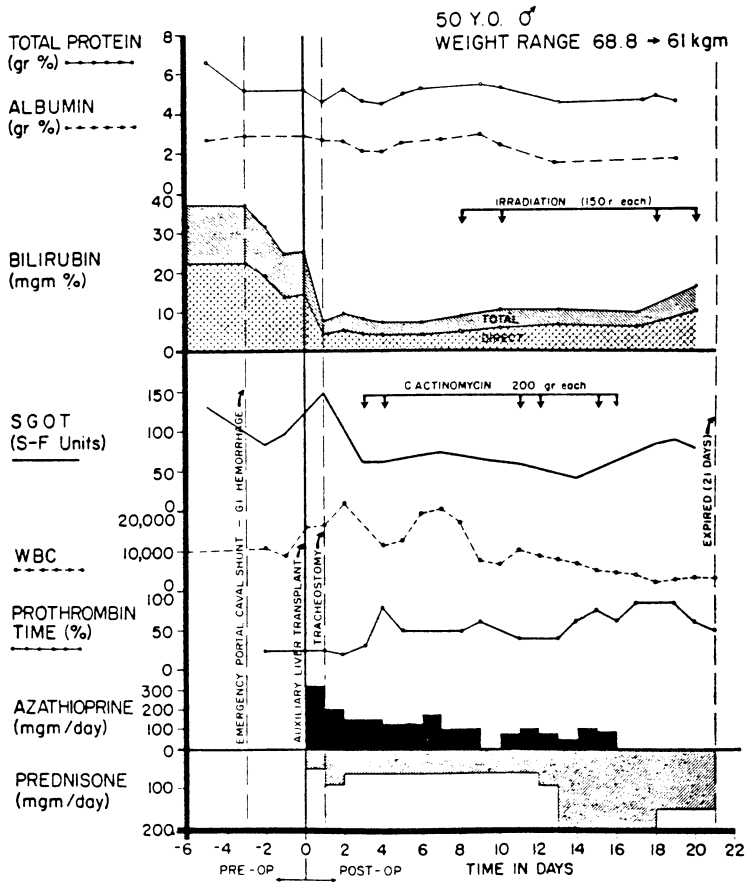

Fig. 5.— Clinical course of patient 1. Note the sharp fall in serum bilirubin after auxiliary homotransplantation, as well as the sustained improvement in prothrombin time. Later, the bilirubinemia slowly increased at a time when rejection was thought to be present. Severe leukopenia ensued late in the second week and necessitated withdrawal of azathioprine.

homograft was grossly well preserved, weighing 1,400 gm. His own cirrhotic liver weighed 1,600 gm. Six liters of ascites were present.

Histologically the portal tracts, particularly the smaller ones, were infiltrated by mononuclear cells (Fig. 6). Between 5% to 10% of the invaders were primitive "blast"-like lymphoid cells with pyroninophilic cytoplasm. Several of the smaller hepatic artery branches showed foci of fibrinoid necrosis in their walls. A few of the larger bile ducts and many of the smaller ducts contained bile casts and there was prominent centrilobular cholestasis. The hepatocytes were depleted of glycogen and the centrilobular cells contained excess lipofuscin and some bile pigment. Those hepatocytes immediately adjacent to the central vein were atrophic. There was also a slight increase in portal connective tissue, and many of the large branches of the hepatic artery showed reduplication of the internal elastic lamina sometimes accompanied by fibroelastic intimal thickening. These latter changes had in all probability occurred in the donor before the liver was transplanted.

Case 2.— A 47 year-old white man had a diagnosis of alcoholic cirrhosis established by percutaneous liver biopsy in November 1964. He was admitted May 28, 1965, with a one-month history of jaundice, anorexia, night sweats, and progressive anemia. He had a large, tender liver, an enlarged spleen, moderate ascites, spider angiomata, palmar erythema, mental confusion, and 2+ guaiac positive stools. The hematocrit reading was 33%; reticulocyte count, 21.3%; total serum bilirubin, 22.2 mg/100 cc with 12.8 mg/100 cc direct; cholesterol, 250 mg/100 cc; total protein, 6.2 gm/100 cc; albumin, 3.10 gm/100 cc; gamma-globulin, 1.28 gm/100 cc; SGOT, 190 SF units; alkaline phosphatase, 9.25 Bessey Lowry units; prothrombin time, 32% increasing to 38% after parenteral phytonadione; fasting blood sugar, 102 mg/100 cc; and BUN, 4 mg/100 cc. During the first month of hospitalization his condition deteriorated with an increase in the serum bilirubin (to 27 mg/100 cc), ascites, abdominal pain, pleural effusion, and the development of fever of unknown origin. After five weeks he suddenly became stuporous prompting consideration of auxiliary homotransplantation.

In preparation for this, a side-to-side portacaval shunt (Fig. 7) and splenectomy were performed on July 4, 1965, at which time he was found to have 5,000 cc of ascitic fluid and portal hypertension. On the following day a cadaveric hepatic homograft was transplanted into his right iliac fossa by a slightly different method (Fig 8) than that used in case 1. The donor was a 12-year-old boy who died from cerebral trauma. Both donor and recipient were 0 blood type. The liver was cooled with cold lactated Ringer's following 40 minutes of external cardiac massage. Arterial revascularization was completed three hours after the donor death, and the portal inflow was completed 45 minutes later.

The immunosuppressive regimen is summarized in Fig. 9, as well as the postoperative liver functions. Bilirubin fell to a low of 4.5 mg/100 cc. A rejection crisis was diagnosed at four days, after which jaundiced deepened. Nevertheless, he was able to resume a full diet and his general condition was excellent until progressive leukopenia developed late in the third postoperative week. Subsequently he had intermittent episodes of fever, gastrointestinal bleeding, and confusion. On the 31st postoperative day he was reexplored for persistent gastrointestinal bleeding; vagotomy and pyloroplasty were performed although a localized site of bleeding was not identified. Terminally he continued to bleed, became anuric and hypotensive, and developed radiographic evidence of bilateral pneumonitis. He died at 34 days.

At autopsy, the patient was shown to have Pneumocystis carinii and cytomegalic inclusion infestation of the lungs. Most of the small bowel and colon were denuded of epithelium secondary to invasive monilial enterocolitis; this was apparently responsible for the hemorrhage. The homograft weighed 1,100 gm; the patient's own cirrhotic liver weighed 1,300 gm. Histologically, there was hemorrhagic necrosis of the centrilobular hepa-

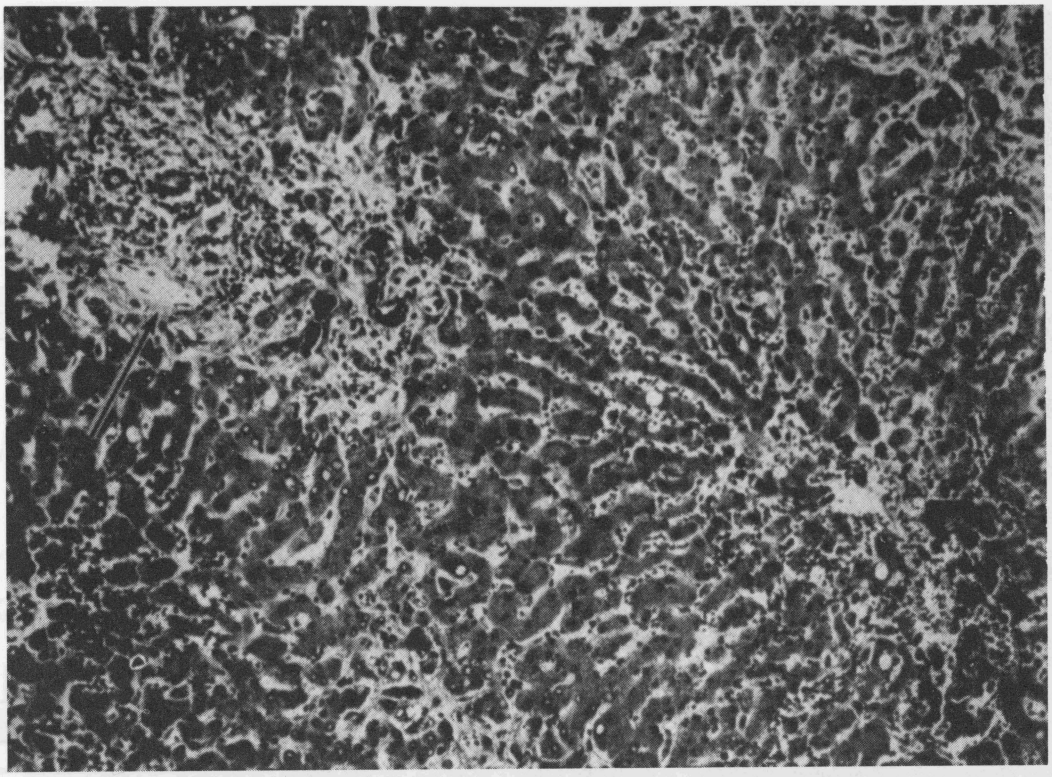

Fig. 6.— Auxiliary human hepatic homograft 22 days after transplantation (case 1). The portal tract (*arrow*) is infiltrated by mononuclear cells and several of the smaller bile ducts contain casts. There is central cholestasis, excess lipofuscin in the central hepatocytes, and atrophy of the liver cells adjacent to the central vein (lower right) (hematoxylin and eosin, X 80).

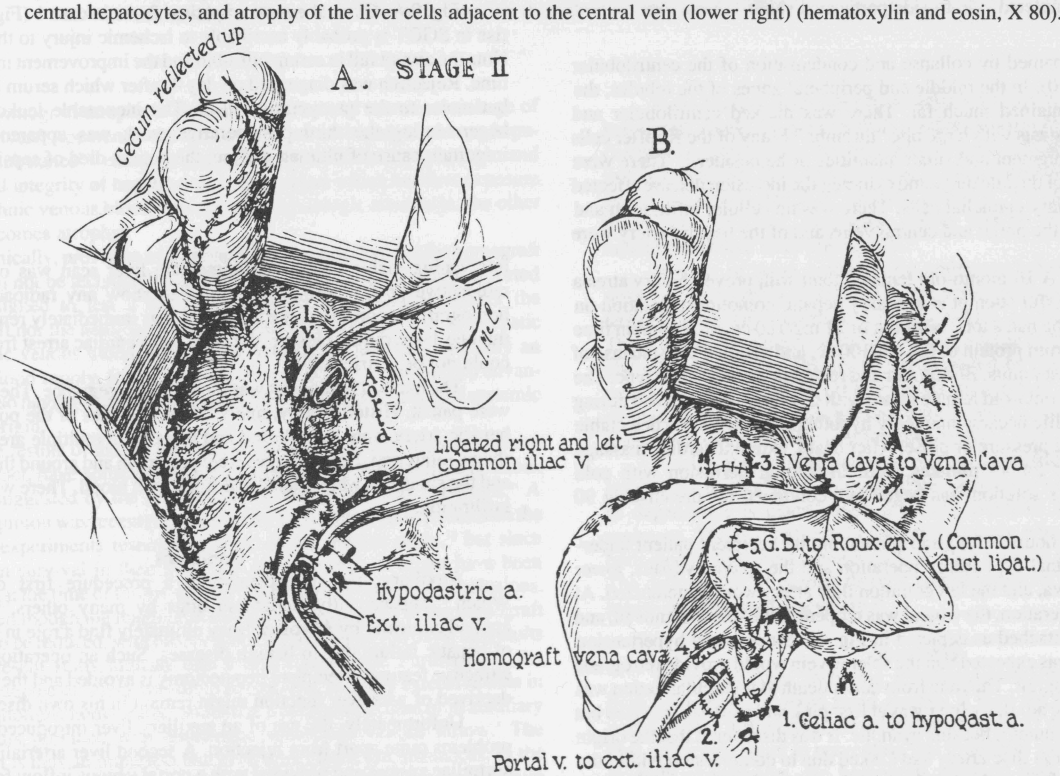

Fig. 8.— Actual auxiliary transplantation (Stage 2). *(A)* Prepared operative field before receipt of homograft. *(B)* Completed operation. Note that the right external iliac vein provides the portal venous inflow. Hepatic venous outflow is into the transected vena cava.

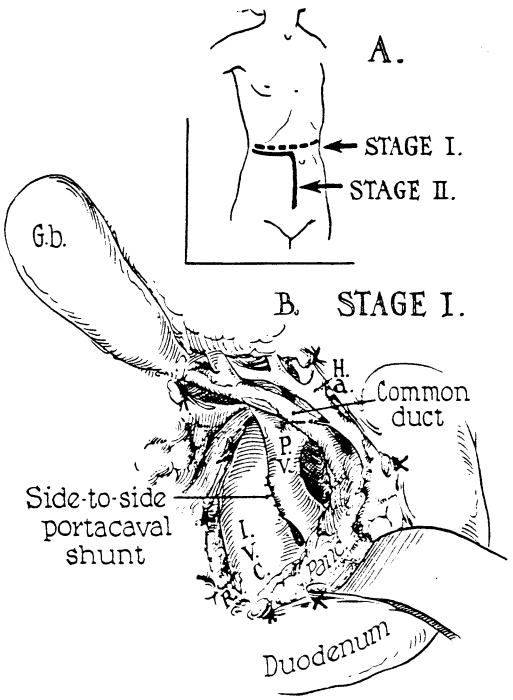

Fig. 7.— Human auxiliary liver homotransplantation. At the first stage, portacaval shunt was performed transabdominally for emergency control of variceal hemorrhage. Stage 2 was the actual transplantation, one day later. *(A)* Incisions: Note that part of the stage 1 incision was reused for stage 2 *(B)* Completed side-to-side portacaval shunt.

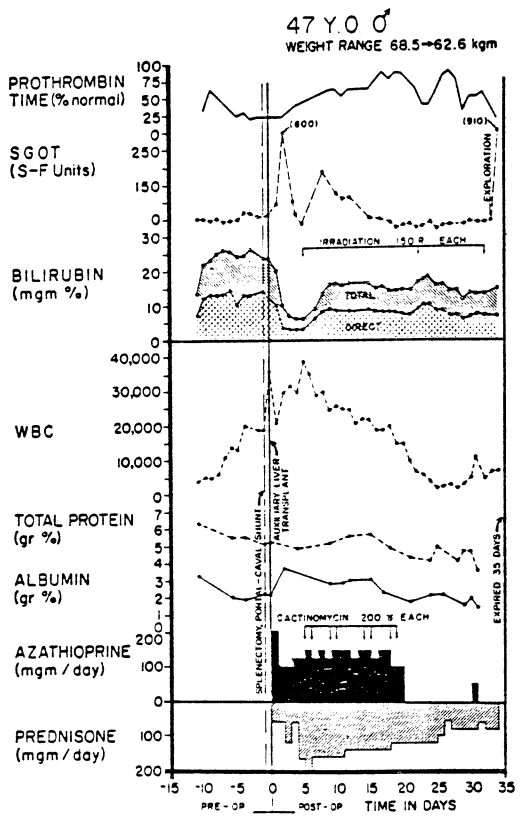

Fig. 9.— Clinical course of patient 2. As in case 1 (Fig. 5) the early rise in SGOT is probably ascribable to ischemic injury to the homograft. Note the abrupt fall in serum bilirubin and the improvement in prothrombin time. Rejection was diagnosed on day 4, after which serum bilirubin rose but never to the preoperative level. The inexorable leukopenia which began during the third postoperative week was apparently the most important cause of ultimate failure; the patient died of sepsis.

tocytes, accompanied by collapse and condensation of the centrilobular reticulin (Fig. 10). In the middle and peripheral zones of the lobules, the hepatocytes contained much fat. There was marked centrilobular and midzonal cholestasis with large bile "thrombi." Many of the Kupffer cells contained bile pigment and small quantities of hemosiderin. There were casts in several of the bile ducts and cytomegalic inclusion disease affected many of the biliary epithelial cells. There was no cellular infiltration and the branches of the portal and central veins and of the hepatic artery were all normal.

Case 3.— A 16-month-old female infant with proved biliary atresia had an unsuccessful attempt at auxiliary hepatic homotransplantation on Nov. 3, 1965. She had a total bilirubin of 21 mg/100 cc, prothrombin time of 70%, total serum protein of 6.8 gm/100 cc, and alkaline phosphatase of 26.4 Bessey Lowry units. Both donor and recipient were A blood type. The donor was a 7-month-old female infant with Crabbe's disease, who during the last hour of life became markedly hypothermic and had no detectable peripheral blood pressure or pulse. After death, external cardiac massage was carried out for 45 minutes, until intraportal perfusion with cold lactated Ringer's solution was begun and continued for the ensuing 90 minutes.

Thirty-six hours before transplantation, the recipient patient underwent an abdominal exploratory operation and the splenic vessels, lower inferior vena cava, and the left common iliac artery were skeletonized. At the definitive operation, the wound was reopened, the spleen removed, and the homograft attached as depicted in Fig. 11. Since portal hypertension was present, it was expected that the splenic vein would provide retrograde flow to the homograft. The time from donor death to revascularization was 153 minutes. The auxiliary liver was at first pink and well vascularized but after about 30 minutes it became cyanotic. It was discovered that the origin of the left common iliac artery had kinked due to compression within the overcrowded abdomen; the distal vessels had clotted. After division of the lowest two lumbar arteries, the arterial reanastomosis was performed. However, the homograft now had multiple cyanotic areas with islands of intervening viable tissue. An immediate liver scan was obtained with radioactive gold (^{198}Au) which failed to show any radioactivity in the homograft. Accordingly, the transplant was immediately removed. Thirty minutes later, during closure, the child had a cardiac arrest from which she could not be resuscitated.

The excised homograft was mottled in appearance. The anastomoses were patent. Histologically, many of the branches of the portal vein and hepatic artery were thrombosed and there were multiple areas of hemorrhagic infarction. Hemorrhages were present in and around the portal areas and the sinusoids were dilated and filled with blood. There was no cellular infiltration.

Comment

Auxiliary liver transplantation, a procedure first described by Welch,[6,7] subsequently studied in dogs by many others,[1,2,8-13] and first applied clinically by Absolon,[14] may ultimately find a role in the treatment of hepatic failure due to benign diseases. Such an operation has certain attractive features. Recipient hepatectomy is avoided and the patient is not deprived of whatever function might remain in his own diseased liver.

Unfortunately, the use of an auxiliary liver introduced physiologic problems quite apart from rejection. A second liver arterialized from the aortoiliac system and provided with a portal venous inflow from the distal vena cava undergoes striking atrophy in the immunosuppressed dog beginning within two weeks.[1] If, alternatively, most of the splanchnic venous flow is diverted through the "heterotopic liver" Marchioro showed

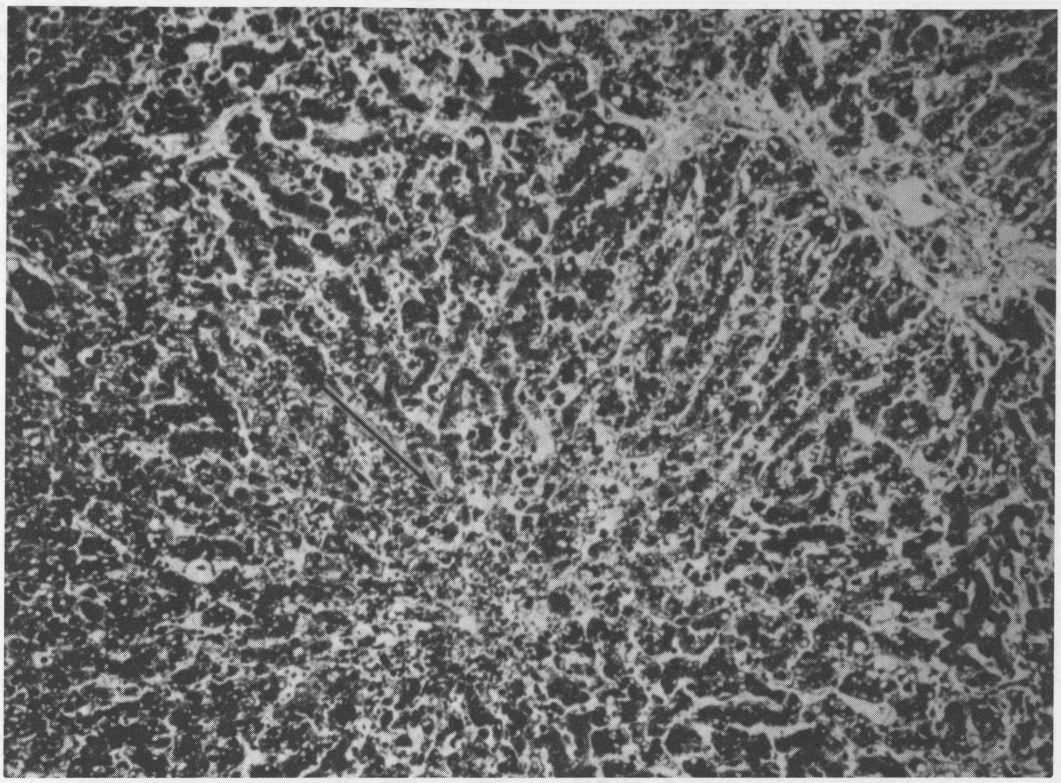

Fig. 10.— Auxiliary human hepatic homograft 34 days after transplantation (case 2). The centrilobular hepatocytes are necrotic (arrow) and there are fat droplets in the liver cells in the midzonal and peripheral parts of the lobule. There is no cellular infiltration (hematoxylin and eosin, X 80).

that the homograft atrophy is prevented and instead there is shrinkage of the host liver.[2] Apparently the splanchnic venous blood contains a hepatotropic substance[3,15] essential for maintenance of the morphologic and functional integrity of hepatic tissue. That organ which has first exposure to splanchnic venous blood operates at a physiologic advantage; the other organ becomes atrophic.

Clinically, provision of a splanchnic venous inflow for the homograft may often not be technically feasible. The animal studies herein reported were designed to test a compromise procedure in which neither the homograft nor the autologous liver was directly perfused with nonhepatic splanchnic venous blood. Instead, the autologous liver received only an arterial blood supply. In contrast, the homograft had the additional advantage of also having a portal venous inflow even though this was of systemic venous origin.

The results of these experiments indicate that host portacaval shunt does, in fact, have some protective affect upon the homograft, a possibility first suggested by the experiments of Marchioro[2] and Thomford.[11] A similar opinion was recently expressed by Tretbar and his associates on the basis of experiments resembling those of the present study,[13] but since maximum survival in their dogs was only 37 days, it might have been argued that the time of follow-up was too short to permit valid conclusions.

Even though the magnitude of the atrophic process in the homograft seemed to be reduced, with observations for as long as 150 days, the results in the present study indicate that it is not prevented. Furthermore, the ultimate degree of injury was often severe, and far more extensive than in either orthotopic homografts[16] studied at a comparable time or in auxiliary homografts provided with nonhepatic splanchnic venous inflow.[2] The operation is thus an imperfect one even under these circumstances. In the three experiments in which both the homograft and the autologous liver had only an arterial supply, the degree of transplant atrophy was even more striking as might be predicted since only the transplant is subject to the ravages of rejection.

In the clinical cases, it might be expected that the environment for the homograft would be even more favorable because of the further loss of competitive potential by the diseased host liver. Whether this was actually true is impossible to say inasmuch as death occurred at too early a time to assess the development of atrophic changes. The degree of histologic injury was, however, somewhat greater than in previously examined human orthotopic liver homografts.[17]

In view of these incompletely understood physiologic considerations, the future value of such auxiliary operations is uncertain. In addition, technical problems constitute a further hazard, as exemplified in case 3. These derive from the necessity of placing a large additional organ into an abdomen which may already be overcrowded by hepatomegaly or splenomegaly. At least eight other attempts at auxiliary hepatic homotransplantation are known to have been made in various centers; in some of these unreported cases, similar mechanical problems were encountered.

Finally, the clinical potential of liver homotransplantation by any heterotopic method or with an orthotopic procedure will be limited as long as dependence is placed upon presently available immunosuppressive regimens. The two patients in the present study who survived operation both died as the direct consequence of drug toxicity and resultant sepsis. The deficiencies of current therapy and suggestions for future improvements are discussed elsewhere.[18]

Summary

In normal dogs, receiving immunosuppressive therapy, auxiliary liver homografts undergo rapid atrophy if they are not provided with a portal venous inflow from the splanchnic bed. In 11 dogs, the influence of host portacaval shunt in preventing this transplant shrinkage was studied. In eight experiments, the homograft received both an hepatic arterial inflow, as well as a portal supply with venous blood returning from the hind quarters; in the other three, only an arterial supply was present. In all 11 dogs, the host liver had solely an arterial supply. The animals were

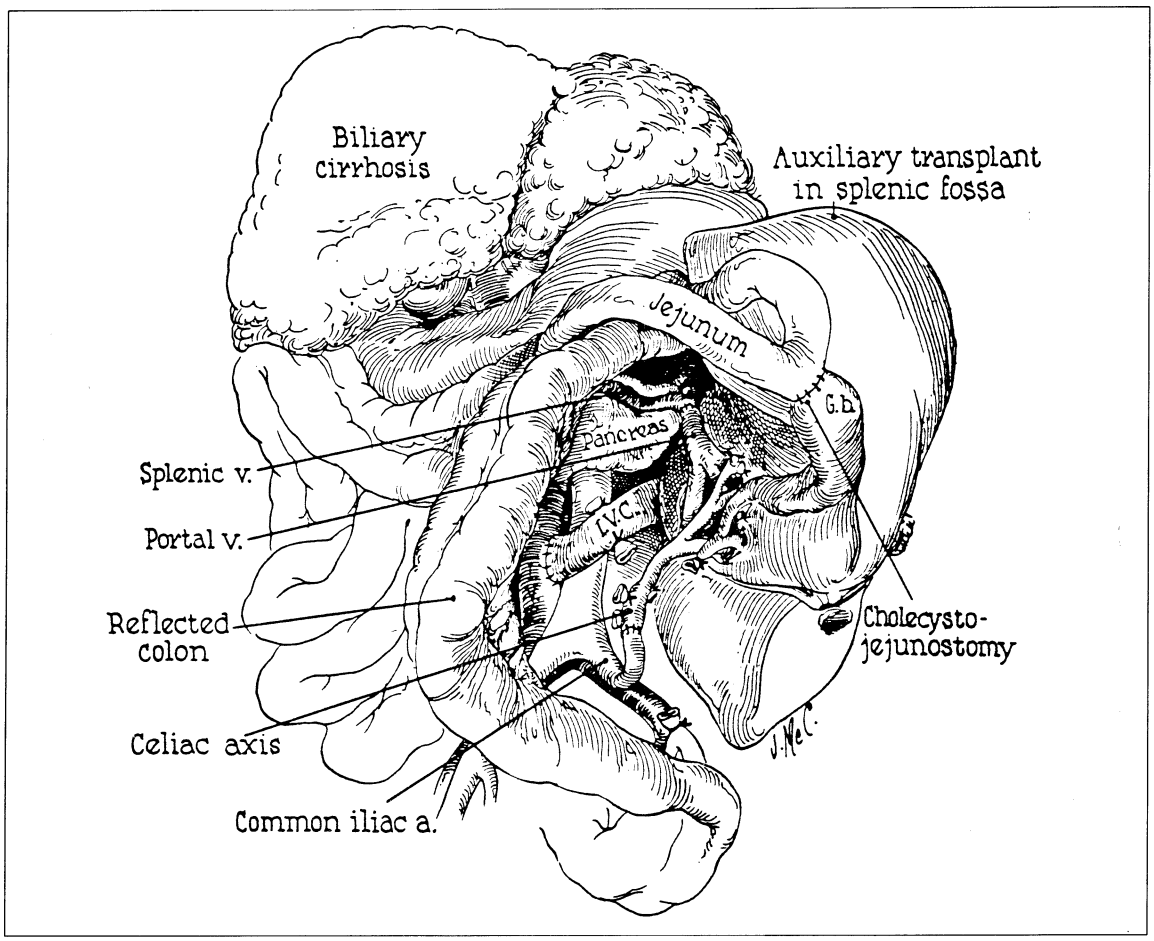

Fig. 11.— Technique used for attempted transplantation in case 3. Note that the homograft is facing medially. The splenic vein and common iliac artery are anastomosed to the homograft portal vein and hepatic artery, respectively. Note also the angulation near the origin of the left common iliac artery. This was responsible for the technical failure of the transplant.

followed for 24 to 150 days.

The results indicate that host portacaval anastomosis has a modest protective effect upon the homograft. Transplant atrophy was partially prevented when the homograft had a double blood supply; it was not influenced when both the homograft and the host liver each had only an arterial supply. In all cases moderate atrophy was observed in the autologous livers, suggesting that the protective effect was at least partly at the expense of injury to the host hepatic tissue. The long-term poor preservation of the homografts emphasize that the operation is not ideal from a physiologic point of view.

Using operations designed to provide the homografts with a physiologic advantage, three attempts at clinical auxiliary transplantation were made. Two adult patients with cirrhosis survived for 22 and 34 days after operation, both eventually dying of sepsis. In these cases, there was unequivocal evidence of homograft function. A third attempt in a child with congenital biliary atresia resulted in technical failure. The specific deficiencies of auxiliary transplantation procedures are discussed.

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Generic and Trade Names of Drugs

Azathioprine — *Imuran*
Prednisone — *Deltasone, Deltra, Meticorten, Paracort*

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Joseph Fortner

This detailed account of a truly successful auxiliary liver transplantation was unique at the time and has been duplicated only once since. Fortner's operation was on 13 December 1972. The portal vein of the auxiliary liver was revascularized from the superior mesenteric vein of the recipient. Fortner studied the interplay between the grafted and host livers, including changes in liver size as rejection or other factors supervened. The clinical observations were explained by the previous animal research on hepatotrophic physiology. The recipient was four years old. He now lives in Phoenix , Arizona and is a junior in high school. Dr. Fortner still practices at the Memorial Sloan-Kettering Cancer Center in New York City, is Professor of Surgery at Cornell University Medical College and President of the General Motors Cancer Research Foundation.

Clinical liver heterotopic (auxiliary) transplantation

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Pessimism regarding clinical auxiliary liver grafts is no longer justified for the liver has been transplanted successfully to a heterotopic position in three patients. Space for the auxiliary liver was achieved by three different methods: pneumoperitoneum, ascites, and abdominal wall reconstruction done with the use of a prosthetic film composed of polyethylene and Marlex. The right paravertebral gutter was found to be a satisfactory site for all three grafts. Splanchnic blood flow was directed preferentially through the donor liver either by ligation of the recipient's superior mesenteric vein proximal to the graft or increased vascular resistance secondary to intrahepatic or extrahepatic host disease. Monitoring the allograft was by clinical evaluation, serum biochemical estimates of liver function, ^{99m}Tc liver scans, and thymidine uptake of cultured peripheral blood lymphocytes. Immune suppression appeared optimal with Cytoxan, low dosage of prednisone, short courses of high-dose methyprednisolone, and high dosage of antihuman lymphocyte globulin (ALG). This type of liver graft seems preferable to the orthotopic graft for selected patients with liver disease.

Liver transplantation to the heterotopic (auxiliary) position can avoid the inherent risks of recipient hepatectomy, especially in patients with noncancerous but fatally damaged livers. In some instances, the recipient's liver might even serve a temporary supportive function during a rejection episode.

The heterotopic (auxiliary) liver allograft poses problems quite different from those of the orthotopically placed graft. These can be classified as mechanical, physiological, and immunological, and they have been studied in three patients at Memorial Sloan-Kettering Cancer Center. All three grafts functioned satisfactorily: two of the three patients survived the early transplantation period; one who survived for eight months has been referred to previously,[6] and one has been discharged from the hospital and is well three months later. One patient died on the thirty-seventh posttransplant day of sepsis from an enteric fistula. Prior to this communication, of 35 patients with a heterotopic graft reported to the ACS/NIH Registry,[1] the longest survivor had lived 34 days.

Case Reports

Case 1. A 72-year-old woman had a localized but unresectable adenocarcinoma involving the common hepatic and intrahepatic bile ducts. Serum total bilirubin was 15.6 mg. percent; conjugated bilirubin was 12.78 mg. percent; alkaline phosphatase was 263 I.U.; serum glutamic ox-

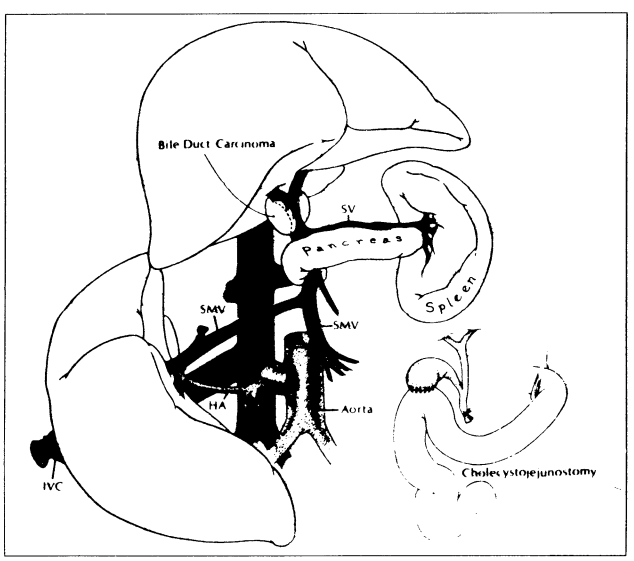

Fig. 1.— Vascular and biliary anastomoses in Case 1. The auxiliary liver allograft was placed in the right paravertebral region. Note bile duct carcinoma constricting recipient portal vein. *HA*, Hepatic artery; *IVC*, inferior vena cava; *SMV*, superior mesenteric vein; *SV*, splenic vein.

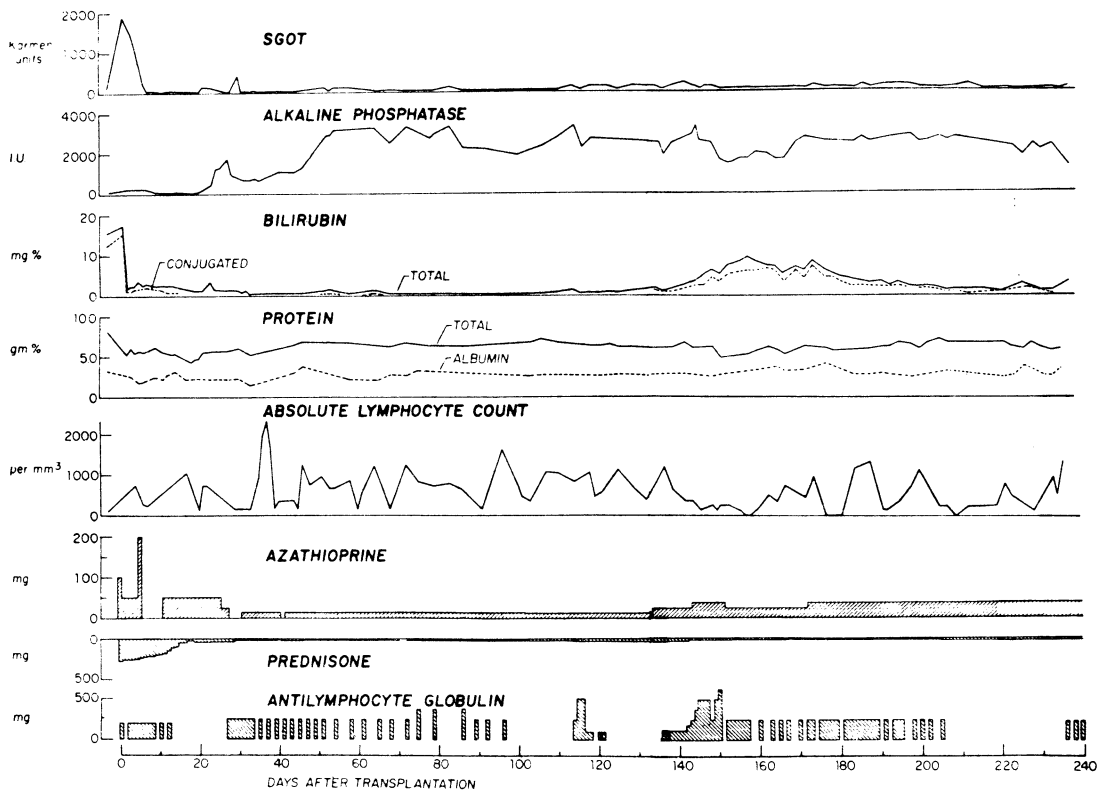

Fig. 2.— Clinical course of Case 1, a 72-year-old woman. Note mild rejection on Day 142 which reversed with increased immunosuppression.

aloacetic transaminase (SGOT) was 117 Karmen units; total protein was 8.3 Gm. percent; and albumin was 3.3 Gm. percent. The auxiliary liver allograft was a palliative measure for uncontrollable itching and jaundice. It was performed on April 26, 1969, with the donor liver coming from a 19-year-old man. Leukocyte typing* with approximately 70 antisera using both microagglutination and microcytotoxicity tests indicated unshared leukocyte antigens to be: donor HL-A 2 and recipient HL-A 5 and 6A. A direct cross-match was negative. Both donor and recipient were ABO group A. The liver was removed from the donor by a technique described elsewhere.[7] After the patient's ascending colon was reflected medially, the graft was placed in the right paravertebral gutter with the dome toward the right flank and its suprahepatic caval stump ligated (Fig. 1). An end-to-side anastomosis was carried out of donor to host vena cava, donor to host superior mesenteric vein, and a segment of donor aorta with attached celiac axis and hepatic artery to recipient aorta. The cold ischemic time was one hour and 55 minutes. The donor had been maintained normotensive until cold perfusion of the liver was started so that there was no warm ischemic time. On establishing blood flow, the liver allograft appeared pink immediately and promptly secreted bile. The gallbladder of the graft was anastomosed to an upper loop of recipient jejunum. The abdominal wall could not be approximated so a synthetic prosthesis composed of an outer layer of Marlex mesh and an inner layer of a sheet of polyethylene was used to bridge the musculofascial gap. Skin closure was accomplished after extensive undermining.

The patient tolerated the procedure well as indicated by a dramatic and sustained reduction in the serum bilirubin levels from preoperative values to 1.6 mg. percent on the second postoperative day. The relentless itching disappeared completely.

During the initial postoperative period, there were a number of serious complications which included severe but temporary central nervous system dysfunction followed by renal failure requiring hemodialysis, massive gastrointestinal bleeding, pneumonitis, septicemia, wound disruption, and congestive cardiac failure. The causes and management of these episodes were complex and bore a direct relationship to the patient's advanced age, basic disease, and the difficulties in closure of the wound. Nevertheless, she recovered from these complications, and good allograft function persisted throughout her protracted convalescence. Mild jaundice was noticed during a late rejection episode 142 days after transplantation which was controlled with increased immune suppression (Fig. 2). Seven months after transplantation, small bowel fistulas developed in the granulating abdominal wound, and she died suddenly as the result of massive gastrointestinal hemorrhage on Dec. 22, 1969, 240 days after transplantation.

At necropsy, the massive bleeding was found to have occurred from a benign gastric ulcer. The patient's own liver weighed 1,250 Gm., was firm and fibrotic, and contained a number of bile ducts filled with inspissated, greenish material. Multiple abscesses were present in the liver parenchyma. At the hilum, scirrhous cancer had invaded and sharply constricted the portal vein. The heterotopic auxiliary liver graft weighed 1,260 Gm. and had a homogeneous glistening red brown appearance with no identifiable areas of necrosis (Fig. 3). The bile ducts appeared normal and all the vascular and the cholecystojejunal anastomoses were patent. Histologically, the liver allograft appeared essentially normal except for sinusoidal dilatation and narrowing of hepatic cords secondary to congestion. There was minimal round cell infiltration in the portal areas. The vessels were normal and patent, and there was no cholestasis. Reticulin stains confirmed the absence of graft atrophy. The cancer had remained localized and there were no metastases.

Case 2. A 42-year-old woman developed hepatitis in March, 1965. Massive hematemesis occurred in October, 1967, and was treated elsewhere by a spleno-renal shunt. There were no further episodes of bleeding.

* Performed at the Leukocyte Typing Laboratory, Department of Serology and Genetics, The New York Blood Center, New York, N. Y.

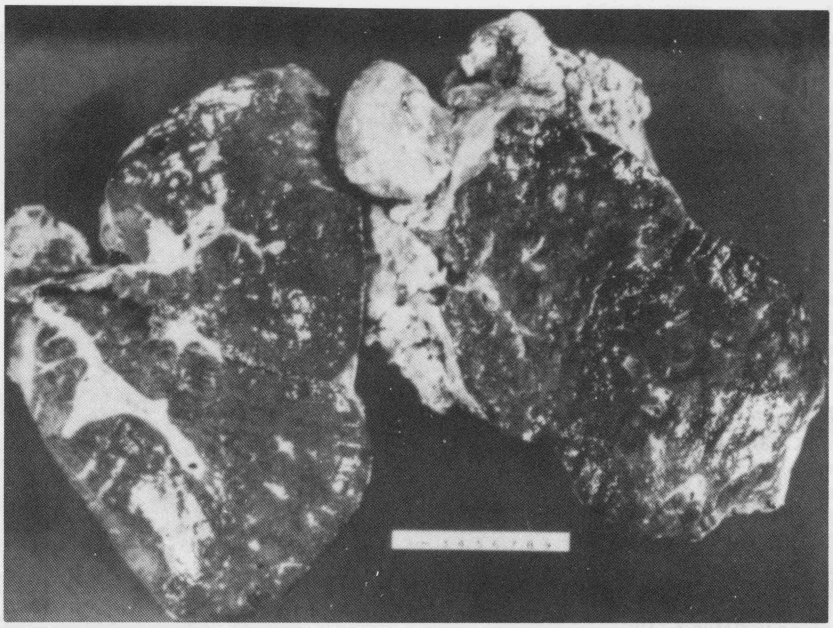

Fig. 3.— The bisected donor liver is on the left; the bisected recipient liver is on the right. Note the similar size of the two livers and normal appearance of the allograft.

On admission to Memorial Hospital on June 23, 1969, a diagnosis of chronic active hepatitis was made, and she was placed on medical management with restriction in both salt and protein intake. The patient did well for more than two years but then required seven hospital admissions over a five-month period because of coma or impending coma. Her last admission was on April 13, 1972, when after recovery on medical management, the risks of future irreversible coma were so great that she remained in the hospital. Just prior to transplantation, serum biochemical values were: total bilirubin, 2.62 mg. percent; alkaline phosphatase, 312 U.; lactic dehydrogenase (LDH), 339 U.; SGOT, 126 U.; total protein, 4.18 Gm. percent; albumin, 2.8 Gm. percent; total cholesterol, 129 mg. percent; and free cholesterol, 37 mg. percent. She was found to have cardiomyopathy and was digitalized. The absence of ascites in this patient caused concern about space for fitting a second liver into the abdomen. Distention of the abdominal wall by pneumoperitoneum was started on May 14, 1972, with 500 c.c. of air being injected. This amount was gradually increased during weekly injections until she was able to tolerate 3,500 c.c. of air without undue discomfort. This was continued until Oct. 28, 1972, when an auxiliary liver transplantation was carried out.

The donor was a 55-year-old woman with ABO blood group O; the recipient was an A. Leukocyte testing with approximately 60 antisera using a microcytotoxicity test revealed no leukocyte antigens in common. A direct cross-match was negative. One-way mixed lymphocyte culture (MLC) studies 2 revealed low stimulation of recipient lymphocytes in reaction mixtures with various concentrations of donor lymphocytes. Reciprocal mixtures showed high stimulation at equal concentrations.

The recipient had a markedly increased collateral circulation, although the portal pressure measured only 150 mm. of water on opening the abdomen. The procedure differed from that described in Case 1 in that the suprahepatic vena cava of the donor was closed by using a continuous No. 4-0 vascular suture (Fig. 4). An end-to-side anastomosis of donor superior mesenteric vein to the larger of the two primary branches of the recipient's superior mesenteric vein was performed. This recipient branch was then ligated cephalad so that splanchnic blood was diverted through the graft. A Carrel aortic patch was used to anastomose donor celiac axis to the recipient's aorta just above the iliac bifurcation. Upon release of the clamps, bile was noted to come promptly from the common bile duct. The end of the common bile duct was ligated, and a No. 12 T tube was inserted into the common duct for external drainage. A cholecystojejunostomy was formed between a loop of jejunum 12 inches from the ligament of Treitz and the junction of the middle and lower thirds of the donor gallbladder. There was severe bleeding from an artery in the gallbladder wall at the time of the anastomosis so that good perfusion of the gallbladder appeared to be present. A gastrostomy was performed, and the abdominal wall was capable of being closed readily without tension. There was no warm ischemic time for the liver. Cold ischemic time established in the recipient, as measured from start of portal vein infusion until blood flow, was one hour and 55 minutes.

Initially, the patient's postoperative course (Fig. 5) was essentially uneventful, and she was eating a soft diet by the seventh postoperative day. Evidence of gastrointestinal fistula developed on the ninth postoperative day which eventually proved to be from an opening in the posterior wall

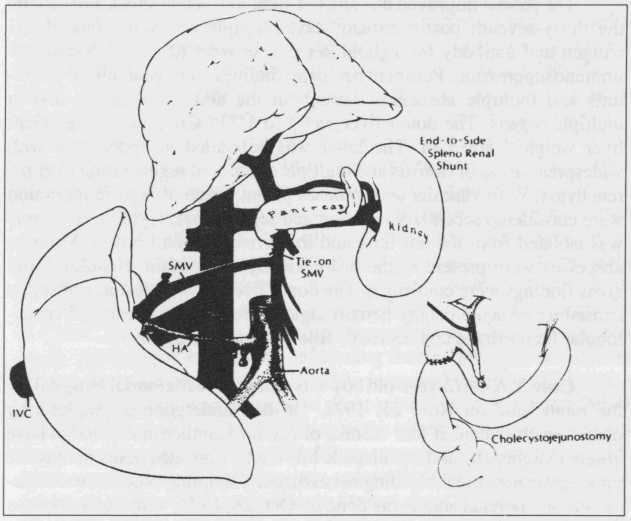

Fig. 4.— Vascular and biliary anastomoses of Case 2. Note that the recipient's superior mesenteric vein has been ligated distal to the graft. See legend to Fig. 1 for meanings of abbreviations.

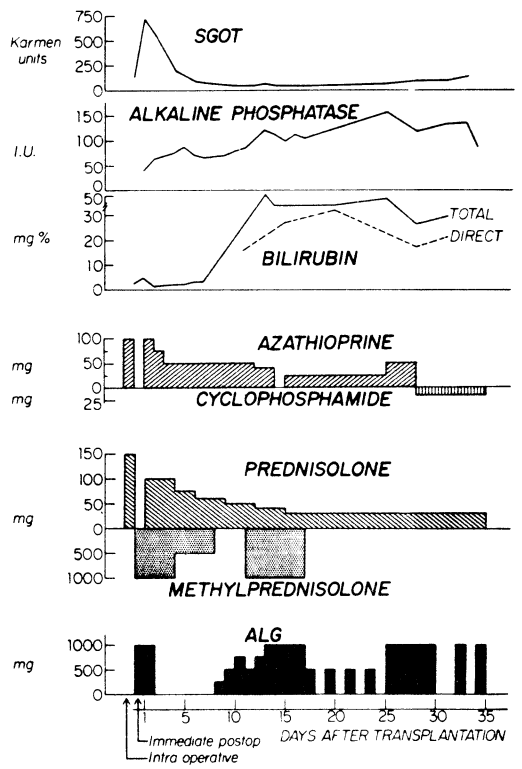

Fig. 5.— Clinical course of Case 2, a 42-year-old woman. Note sharp elevation of bilirubin at Day 10 which was likely secondary to mechanical biliary tract obstruction and sepsis. ALG was withheld early because of thrombocytopenia.

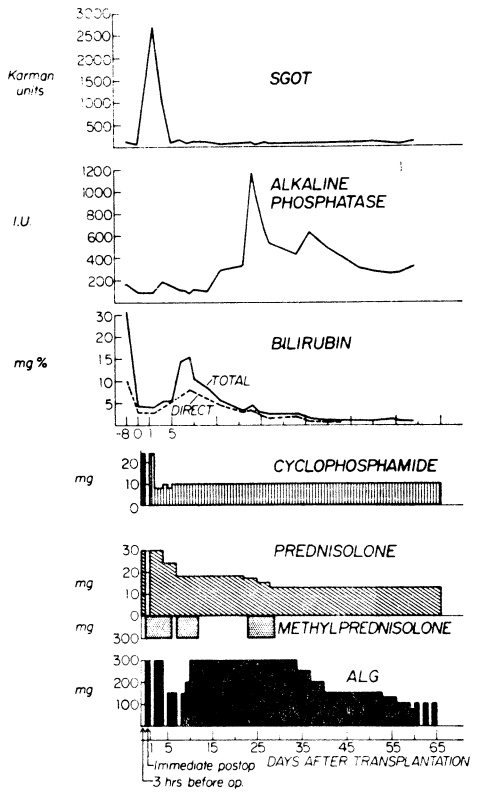

Fig. 6.— Clinical course of Case 3, a 5-year-old boy. Note abnormal liver chemistries on the twenty-fourth day, suggesting mild rejection which was reversed.

of the gallbladder about 2 cm. away from the cholecystojejunostomy. The patient became increasingly icteric and septic, requiring two laparotomies for drainage, biliary diversion, and gastrointestinal repair. The donor liver appeared bile stained but otherwise normal in appearance and consistency at these operations.

The patient improved initially but went into septic shock and died on the thirty-seventh post-transplant day. Multiple determinations of SH antigen and antibody throughout her course were negative, despite the immunosuppression. Pertinent necrospy findings were generalized peritonitis and multiple abscesses throughout the abdominal cavity and in multiple organs. The donor liver weighed 1,725 Gm., and the recipient liver weighed 800 Gm. The latter was hobnailed in appearance with widespread areas of fibrosis and multiple nodules of regenerating liver parenchyma. With vascular anastomoses patent, areas of hepatic infarction were considered secondary to prolonged septic shock. Cytomegalic virus was isolated from the left lung and the serosa of small bowel. Monilial abscesses were present in the heart, kidneys, and brain. Histologically, gross findings were confirmed. The donor liver showed features of sepsis consisting of acute patchy hemorrhagic necrosis and widespread centrilobular hemorrhage and necrosis. Bile stasis was present.

Case 3. A 4-1/2-year-old boy was admitted to Memorial Hospital for the ninth time on Nov. 21, 1972. He had undergone an exploratory operation elsewhere at four months of age for jaundice and found to have absent extrahepatic and intrahepatic bile ducts. Repeated bouts of massive upper gastrointestinal bleeding necessitated a shunting procedure. A side-to-side splenorenal shunt was done on Oct. 28, 1971, with ligation of the splenic artery but without splenectomy, dropping the portal pressure from 480 to 320 mm. H_2O (mesenteric vein). There was no further gastrointestinal bleeding for several months, but his general condition slowly deteriorated. Ascites developed, pruritis was severe and unrelenting, jaundice was marked, and there was splenomegaly. Massive gastrointestinal bleeding occurred in October and again in November, 1972, ceasing spontaneously just prior to transplantation.

Representative of his serum values prior to the most recent bleeding episode were: total bilirubin, 34 mg. percent; direct bilirubin, 23 mg. percent; alkaline phosphatase, 284 U.; LDH, 252 U.; SGOT, 228 U.; total protein, 5 Gm. percent, albumin, 3.1 Gm. percent. A ^{99m}Tc liver scan showed a small liver measuring 14 by 13 cm., with very poor isotope uptake.

On Dec. 13, 1972, a 12-year-old male donor became available. The donor was ABO type O and the recipient was A. Leukocyte typing using about 60 antisera in microcytotoxicity testing revealed no leukocyte antigens to be shared. One-way MLC testing[2] showed similar values to those obtained in Case 2 for the mixture in which recipient cells served as responders. There was minimal activity in the reciprocal mixture, however.

Removal of the donor liver was as described previously.[7] The recipient's markedly distended abdomen contained about 2,000 c.c. of clear ascitic fluid. Portal pressure prior to placement of the allograft was 380 mm. H_2O and at the end of the procedure, portal pressure was 300 mm. H_2O. The auxiliary liver was placed in the right paravertebral gutter as described for Case 2 with some modifications. Donor superior mesenteric vein was anastomosed, end to side, to the largest main branch of the recipient's superior mesenteric vein. The latter was not ligated, however. The gallbladder was removed. A Roux-en-Y limb of upper jejunum, 25 cm. in length, was fashioned and an end-to-side choledochojejunostomy constructed. A No. 12 T tube was placed in the common bile duct. A gastrostomy was made and the abdomen was closed with tension which was judged to be acceptable. The volume of donor liver was approximately

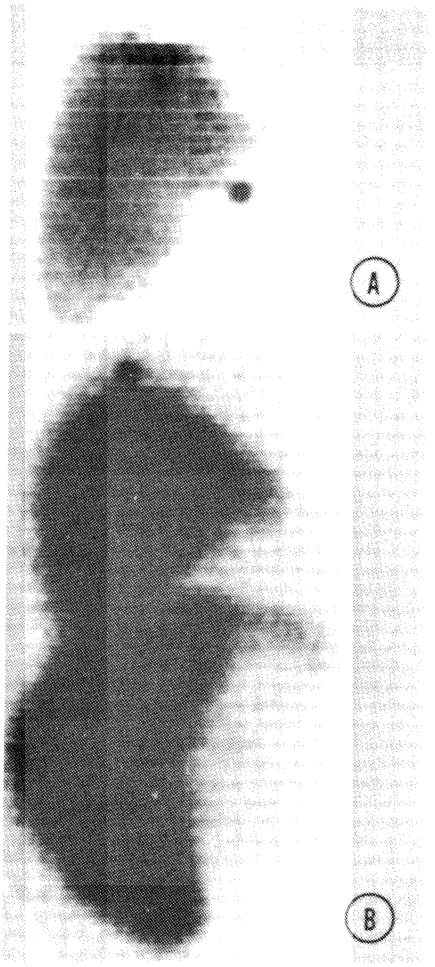

Fig. 7.— Liver scans (^{99m}Tc sulfur colloid) preoperatively (*A*) and six months after transplantation (*B*) in Case 1. Note uniform uptake in allograft (*B*, lower portion) which shows no evidence of atrophy.

that of the 2,000 c.c. of ascitic fluid which had been present. The cold ischemic time was one hour and 45 minutes. There was no warm ischemic time.

His postoperative course was uneventful (Fig. 6). The poor nutritional status of the recipient caused us to place him on intravenous hyperalimentation on the second postoperative day. This was discontinued on the eighteenth postoperative day. The gastrostomy was occluded on the eighth postoperative day, and oral feedings were started on the fourteenth postoperative day. He has been on a regular diet since the twenty-first posttransplant day, active and showing marked development in personality and strength. The T tube was removed on the fifty-fourth postoperative day, and he was discharged from the hospital on the sixty-ninth posttransplant day with a total bilirubin of about 1 mg. percent.*

Mechanical Features

The fitting of a second liver into an abdomen can be difficult. Abdominal wall closure was possible in Case 1 only by using synthetic material. Breakdown of the wound occurred and was the source of many complications throughout the patient's eight-month posttransplantation survival period. An ideal setting existed in Case 3 in whom the presence of ascites and the availability of a small donor liver made closure of the abdominal wall possible with primary healing of the wound. Space limitations created by the absence of ascites in Case 2 were compensated for by using pneumoperitoneum. This procedure was most satisfactory and created adequate space for the auxiliary liver.

Placement of the allograft in the right paravertebral region was chosen for all three patients. This site seems desirable because of its contour, the ease with which it can be prepared to receive the graft, and its proximity to structures essential for establishing the transplant. This arrangement is similar to that which Bengoechea-Gonzalez and associates[4] used in canine studies. Other authors[5,15,17] have placed the graft in the pelvis, central abdomen, splenic bed, or either flank in both clinical and experimental auxiliary liver transplantation. Clinical failure has occurred in some cases because of kinking of blood vessels or insufficient space.

Physiological Considerations

Vascular circuitry for the grafts is indicated in Figs. 1 and 4. Experimental studies have indicated that prevention of auxiliary liver graft atrophy is a major problem.[11,12,15,19] A variety of physiological factors[11,12,17,20,21] appear to influence the integrity of the auxiliary graft but optimal conditions for the graft appear to be met when there are alterations in splanchnic venous blood flow so that the transplanted liver receives blood preferentially to the recipient's liver. Bile duct obstruction of the host liver seems to potentiate this.[3,9,14] Case 1 presented such a situation: a natural pathological setting of the cancer completely blocking the extrahepatic bile ducts as well as constricting, selectively, the portal vein. Graft atrophy was avoided and a homeostatic balance was achieved by the donor and recipient livers as indicated by their similarities at necropsy (Fig. 3). Preoperative and six-month postoperative liver scans (^{99m}Tc sulfur colloid) are shown in Fig. 7. There was no evidence of graft atrophy during the entire posttransplant period.

Liver cell dysfunction provided an advantage for the graft in Case 2. Extrahepatic biliary tract obstruction was absent, and the portal pressure was apparently normal. A splenorenal shunt had been done previously with apparent decompression of the splanchnic bed. In order to obtain preferential splanchnic blood flow through the donor liver, ligation was done of the anastomosed primary branch of superior mesenteric vein on its cephalad side (toward the recipient's liver). Increased distension of the graft superior mesenteric vein was noted immediately after this ligature was placed. There was no evidence of vascular compromise of the intestine. Atrophy of the graft was not apparent during its 37 day survival. Serial ^{99m}Tc sulfur colloid liver scans were performed immediately before transplantation and serially thereafter (Fig. 8). There were no changes evident in size of the auxiliary liver, maximal counting rates, or in the relationship between uptakes in the auxiliary liver and marrow. Dynamic studies of sulfur colloid uptake in the donor liver at various intervals were also carried out. These showed lower maximal counting rates of the auxiliary liver and a slower rate of initial slope of liver uptake on the seventeenth day, suggesting rejection or some other abnormality (Fig. 9). Uptake values rose to a maximum by the twenty-fourth day. The presence of sepsis, severe hemolysis, and biliary tract obstruction makes interpretation of these findings difficult. Apparent improvement in radiocolloid uptake coincided with treatment for suspected rejection and drainage of a retroperitoneal abscess. These preliminary observations demonstrate a good blood supply to the graft as indicated by satisfactory colloid uptake and may be useful in differentiating various complications encountered in liver transplants.

Biliary atresia and a markedly elevated portal pressure were present in Case 3. A previous side-to-side splenorenal shunt was of questionable patency since repeated episodes of bleeding from esophageal varices were present just prior to transplantation. The increased vascular resistance in the recipient's liver would appear to shunt splanchnic blood preferentially through the donor liver. Distal ligation of the superior mesenteric vein was therefore not performed. Sequential liver scans were obtainable from the fifth posttransplant day to the seventy-second day (Fig. 10). These revealed a decrease in size of the patient's own liver of about 30 percent on the fifth day with a further decrease to about 50 percent of original size during the latter part of the study. Slow but steady decrease in spleen size was noted also, with a 20 percent reduction about the twentieth posttransplant day and

* The patient is presently living and well nine months after transplantation with a serum total bilirubin of 0.6 mg. per cent.

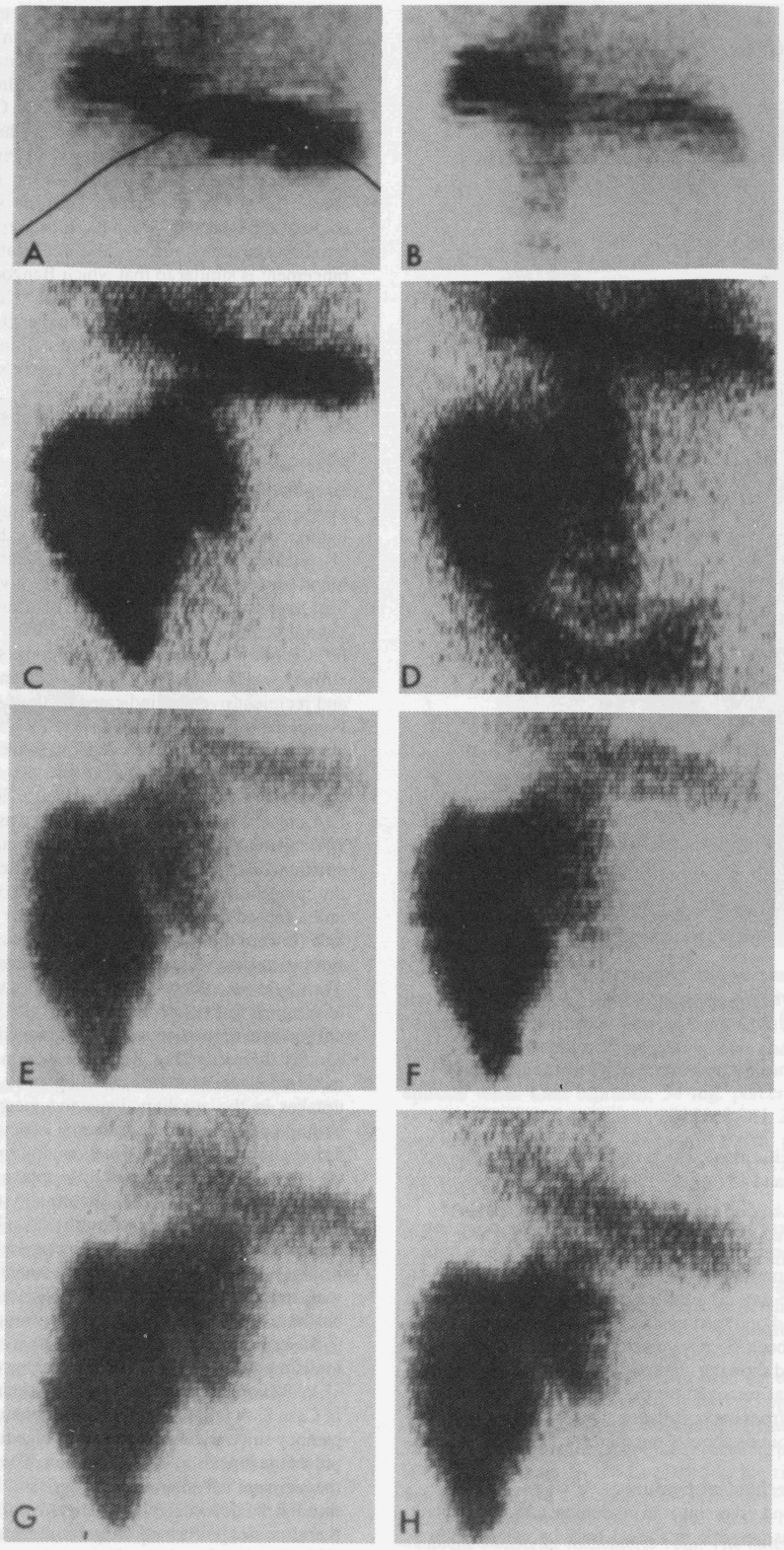

Fig. 8.— ^{99m}Tc sulfur colloid liver scans of Case 2. Note good uptake of donor liver throughout her course and the small size of her own liver. *A*, Preoperative anterior scan. *B*, Preoperative posterior scan. *C*, Third posttransplant day, anterior scan. *D*, Third posttransplant day, posterior scan. *E*, Eleventh posttransplant day. *F*, Eighteenth posttransplant day. *G*, Twenty-first posttransplant day. *H*, Twenty-eighth posttransplant day.

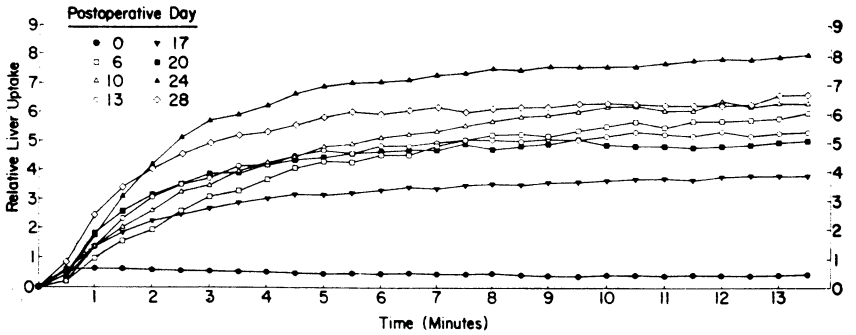

Fig. 9.— Dynamic studies of ^{99m}Tc sulfur colloid uptake by transplant liver. Relative counting rates of the transplant liver were calculated with the use of the base line of uptake obtained on the sixth posttransplant day from immediately after injection to 15 minutes.

a 30 percent reduction by the sixty-fifth day. The radioactivity in the spleen was 2.6 times that of the auxiliary liver on the fifth post-transplant day. By the twenty-third day, the maximal counting rate of the spleen was similar to that of the auxiliary liver. Subsequently, it has decreased even further so that the maximal counting rate in the auxiliary liver exceeds the splenic uptake. This suggests improved reticuloendothelial function of the grafted liver and possible shunting of blood to the grafted liver away from the spleen. These findings support the thesis that increased vascular resistance in the recipient's liver has diverted splanchnic blood through the donor liver preferentially. The significance of increases of maximal liver uptake on the twenty-third and fifty-first day is not clear, although there was some clinical evidence of mild allograft rejection during the fourth posttransplant week. The radionuclide in the liver has remained homogeneous, and the relationship between uptake of auxiliary liver and spleen has remained satisfactory. On the fifth posttransplant day, the transplanted liver measured 18 cm. high and 15 cm. wide with a surface area of 216 cm.^{2} On the fifty-eighth postoperative day, the liver measured 19 by 11 cm. with a 194 cm.^{2} surface area. These differences are not significant.

Immunological Considerations

Monitoring the auxiliary liver graft has been by clinical evaluation, serum biochemical estimations of liver function, serial ^{99m}Tc liver scanning, and testing of in vitro thymidine uptake of peripheral blood lymphocytes. Results of liver function tests must be evaluated with the use of abnormal pretransplant values as a base line. Elevation in the alkaline phosphatase, LDH, SGOT, and 5' nucleotidase, for example, may be secondary to changes in the recipient's liver rather than to any abnormalities in the auxiliary graft. Serial changes rather than absolute values are the important features. Serum bilirubin levels appear to be especially useful in detecting rejection, although its elevation may be due to other causes.

We have found spontaneous in vitro ^{3}H-thymidine incorporation by blood leukocytes to be a useful corroboration test. A modification of the technique of Hersh and associates[10] was used. Thymidine uptake was sharply increased on the thirteenth posttransplant day in Case 3 (Fig. 11). Clinical and biochemical signs of rejection were not seen until the twenty-third posttransplant day, when a sharp elevation in serum alkaline phosphatase and some elevation in bilirubin levels developed. This was treated by a six-day course of a daily bolus of 300 mg. of methylprednisone given intravenously. There was a gradual reversal of these abnormalities. By the forthy-first posttransplant day, there was no clinical or biochemical evidence of rejection, and normal levels of spontaneous leukocyte reactivity were found. These findings continue to the present. The presence of sepsis and severe hemolysis in Case 2 made cell culture studies difficult to interpret. Lymphocyte reactivity was at a normal level through the tenth posttransplant day, during which time there was no clinical or biochemical evidence of rejection. Enteric fistula and sepsis then developed. Lymphocyte testing thereafter showed a sharp increase in immediate reading which could be ascribed to sepsis. Lymphocyte reactivity studies were incomplete in Case 1.

Immunosuppression was by equine antilymphocyte globulin,* steroids, and azathioprine† for the first two patients. Cyclophosphamide (Cytoxan) was used instead of azathioprine in Case 3 (Fig. 6). Recent data from our laboratory,[8] as well as that of others[13,18] indicate cyclophosphamide is preferable to azathioprine in cases in which liver dysfunction may exist. Dosage for Cytoxan was as recommended by Starzl and associates[18] for orthotopic liver transplants. Prednisone has been used in lower doses for Cases 2 and 3 than previously[6] because of the potential of fatal infectious complications. The diminished dosage has been compensated for by using large doses of methylpredniosolone (1 Gm. in adults; 20 mg. per kilogram in children) given intravenously daily for the first five posttransplant days. This dosage has been repeated without any sign of rejection. Equine antilymphocyte globulin has been given at unusually high dosage levels particularly for the most recent patient (Case 3).

Conclusion

Clinical liver heterotopic (auxiliary) allografts function well when splanchnic blood flow is directed preferentially to them. Functional competition appears to exist between donor and recipient livers. A balanced state seems to have been achieved in the first patient where donor and recipient liver weighed essentially the same after eight months. Progressive shrinkage of recipient liver and spleen on serial scanning of the third patient during the initial posttransplant period supports this thesis. Vascular circuitry must be individualized according to the recipient's pathology. Steps must be taken to achieve preferential splanchnic blood flow for the transplant if portal hypertension does not exist.

Differentiation of rejection from other possible complications remains difficult. Immune suppression has been improved by substituting Cytoxan for azathioprine, more judicious use of steroids, and improved ALG given at high dosage. Further clinical use of the heterotopic (auxiliary) liver allograft is indicated.

We are indebted to Dr. Schwick and Dr. Heide for their generosity in making Behringwerke ALG available.

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* ALG obtained from two sources: University of Minnesota (MAG); and donated by Behringwerke AG.

† Supplied by Burroughs-Wellcome, Inc. Research Triangle Park, N.C.

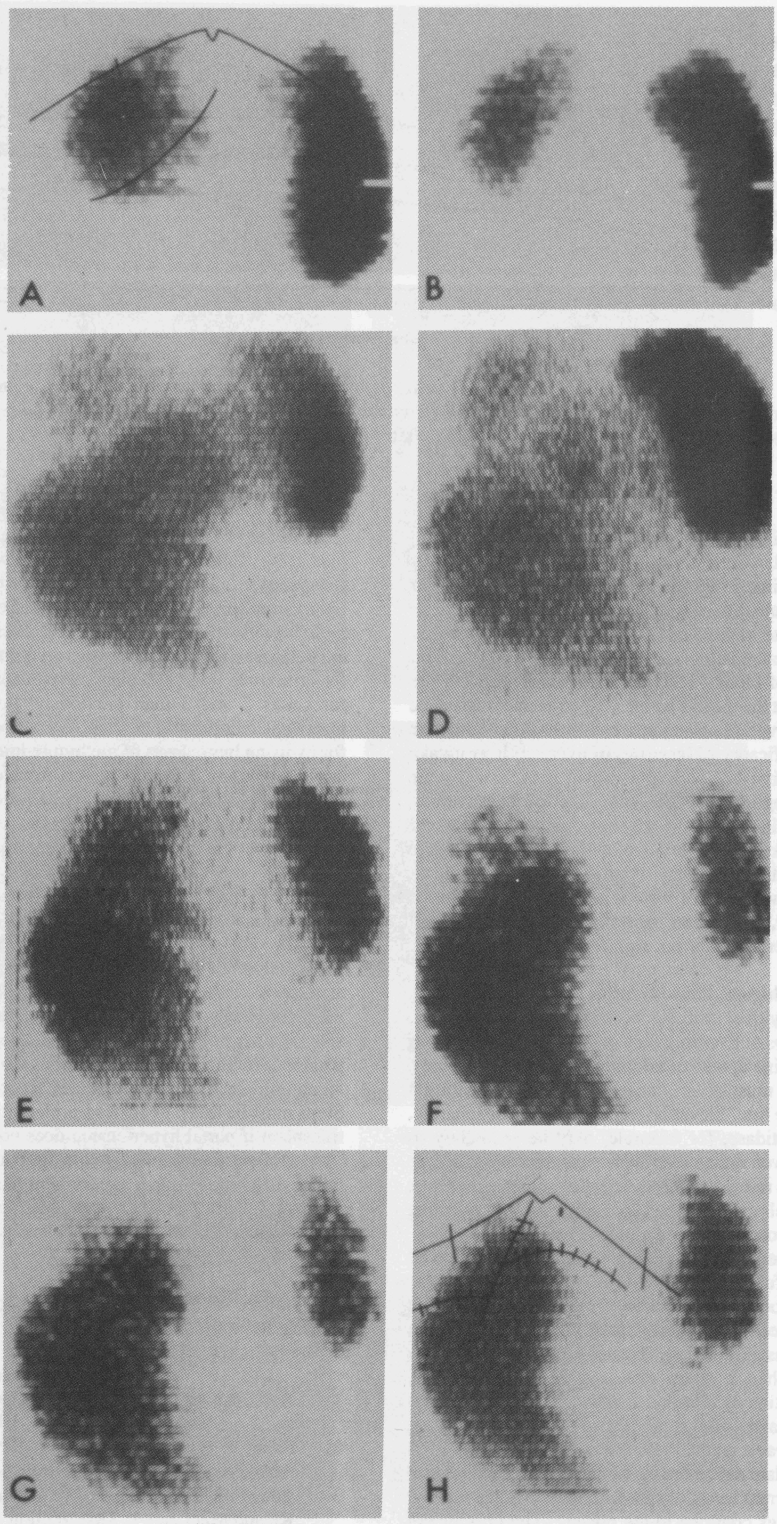

Fig. 10.— Sequential ^{99m}Tc sulfur colloid liver scans on Case 3. *A*, Preoperative anterior scan. *B*, Preoperative posterior scan. *C*, Fifth posttransplant day, anterior scan. *D*, Fifth posttransplant day, posterior scan. *E*, Twenty-seventh posttransplant day. *F*, Fifty-eighth posttransplant day. *G*, Sixty-fifth posttransplant day. *H*, Seventy-second posttransplant day. Note changes in relative sizes of spleen and recipient liver. The latter appears to be sharply decreased in size from the preoperative period.

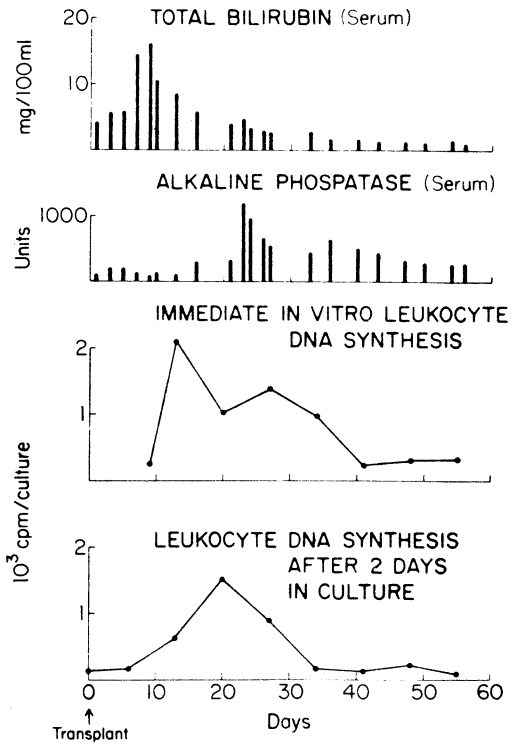

Fig. 11.— In vitro tritiated thymidine incorporation by peripheral blood lymphocytes is correlated with serum bilirubin and alkaline phosphatase values in Case 3. Mild rejection reaction may have been predicted by the leukocyte studies.

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Shunzaburo Iwatsuki

Epilogue

Between March, 1980 and July, 1987, 1,000 patients underwent liver replacement under cyclosporine therapy by the team that originally had worked at the University of Colorado. This experience was preceded by 170 patients who received liver grafts from 1963-80 with azathioprine and steroid therapy, with or without ALG. A report of this total experience was presented by Shunzaburo Iwatsuki at an International Transplant Meeting in Pittsburgh in September, 1987.

Dr. Iwatsuki was born in Nagoya, Japan in 1940. He trained in the United States in hepatology and in surgery after already having been fully trained in his native country. He became a fellow in transplantation at the University of Colorado from 1971-3 and returned for an additional transplantation fellowship in 1979-80. Iwatsuki became one of the most extraordinarily well-trained and experienced surgeons in the world. He became a major force in developing the field of liver transplantation and, of equal importance, in training other surgeons who have proliferated liver transplant technology all over the world.

Experience in 1,000 liver transplants under Cyclosporine-steroid therapy: A survival report

Transplantation Proceedings, 20: 498-504, 1988

S. Iwatsuki, T. E. Starzl, S. Todd, R. D. Gordon, C. O. Esquivel, A. G. Tzakis, L. Makowka, J. W. Marsh, B. Koneru, A. Stieber, G. Klintmalm and B. Husberg

Since the first human liver transplantation in Denver on March 1, 1963, numerous refinements in surgical techniques and in perioperative care have been made, the mechansim of graft rejection has been more precisely understood, and safer and more effective immunosuppressive agents have been discovered.[1-3] Now, in 1987, it is well accepted that liver transplantation is often the only effective therapy for many advanced liver diseases.

We will report here, as a landmark of our continuing efforts, the survival of patients with various end-stage liver diseases after liver transplantation from our own experience in 1,000 hepatic homograft recipients under cyclosporine-steroid therapy.

Case Materials and Methods

Since the introduction of cyclosporine-steroid therapy for our liver recipients in March 1980, 1,000 patients received liver homografts for various end-stage liver diseases by July 1987 at our institutes in Denver (in 1980), in Pittsburgh (since 1981), and Dallas (since 1985).

Of the 1,000 liver recipients, 666 were adults of age 18 years or older, and 334 were children of the ages younger than 18 years. The youngest liver recipient was 26 days old, and the oldest one was 76 years old at the time of transplantation. The numbers of patients in various age groups are listed in Table 1.

The liver diseases of adult recipients and those of pediatric recipients

Table 1. Number of Patients in Various Age Groups, and 1- to 5-Year Survival Rates

Age (yr)	No. Patients	Actuarial Survival (%)				
		1 year	2 years	3 years	4 years	5 years
0-1	30	63	55	49	37	37
1-2	40	66	66	66	66	66
2-3	48	70	68	65	65	65
3-4	35	71	68	68	68	68
4-5	29	58	58	58	58	58
5-10	84	78	74	72	72	68
10-18	68	80	79	76	76	76
18-30	128	77	73	66	58	58
30-40	160	77	75	70	64	60
40-50	209	75	73	70	70	70
50-60	121	76	70	61	61	61
60-70	47	59	56	56	—	—
70 and older	1	100	—	—	—	—

Table 2. Liver Diseases of Adult Recipients

Disease	No. Patients
Cirrhosis (postnecrotic, cryptogenic, alcoholic)	278
Postnecrotic and cryptogenic	237
HB_sAg positive	36
Alcoholic	41
Primary biliary cirrhosis	165
Primary sclerosing cholangitis	74
Liver-based inborn metabolic errors (Alpha-1-antitrypsin deficiency, Wilson's, etc)	35
Primary hepatic malignancy	33
Fulminant hepatic failure	27
Secondary biliary cirrhosis	13
Budd-Chiari syndrome	13
Bile duct cancer	10
Secondary hepatic malignancy	7
Others*	11

*Cystic fibrosis (3), adenomatosis (2), biliary atresia, cryptococcal cholangitis, congenital hepatic fibrosis, polycystic disease, trauma, lymphoangiomatosis.

are listed in Table 2 and Table 3, according to the incidence. Three most common liver diseases among adult recipients were (1) postnecrotic cirrhosis (including chronic active hepatitis and cryptogenic cirrhosis), (2) primary biliary cirrhosis, and (3) primary sclerosing cholangitis. Those of pediatric recipients were (1) biliary atresia (including extrahepatic and intrahepatic type, biliary hypoplasia and Alagille's syndrome), (2) liver-based inborn metabolic errors (alpha-1-antitrypsin deficiency disease, Wilson's disease, tyrosinemia, and others), and (3) postnecrotic cirrhosis.

The survival data were analyzed as of September 1, 1987, using the method of Kaplan-Meier. The statistical comparisons were made by the methods of Breslow and of Mantel-Cox. The difference was considered as significant when the *P* value was less then .05.

Results

Overall survival rates of the 1,000 recipients treated with cyclosporine-steroid therapy were three times higher than those of 170 patients treated with azathioprine-steroids before 1980. One- to 5-year survival rates were 74%, 71%, 67%, 65%, and 64%, respectively, as shown in Fig. 1. Five percent to 10% survival superiority of the pediatric recipients had existed for pediatric recipients during the azathioprine era

Table 3. Liver Diseases of Pediatric Recipients

Diseases	No. Patients
Biliary atresia	179
Liver-based inborn metabolic errors (Alpha-1-antitrypsin deficiency, Wilson's, etc)	63
Cirrhosis (postnecrotic and cryptogenic)	39
Familial cholestatic syndrome	15
Fulminant hepatic failure	13
Secondary biliary cirrhosis	7
Congenital hepatic fibrosis	7
Primary hepatic malignancy	3
Budd-Chiari syndrome	2
Neonatal hepatitis	2
Others*	4

*Primary sclerosing cholangitis, trauma, focal nodular hyperplasia, inflammatory pseudotumor.

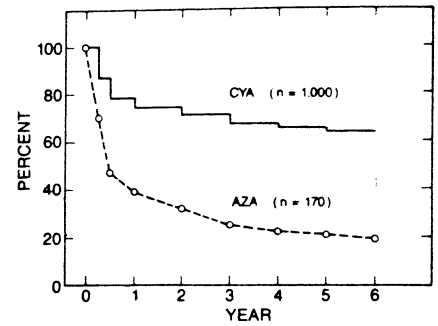

Fig. 1.— Overall actuarial survival rates of 1,000 patients treated with cyclosporine-steroid therapy in comparison with overall actual survival rates of 170 patients treated with azathioprine-steroid therapy.

before 1980. Since 1981, the survival rates of adult and of pediatric recipients are identical under cyclosporine-steroid therapy (Figure 2).

The survival rates of various age groups of recipients were compared in Table 1. The survival rates of children under the age of 1 year and those of adults aged 60 years or older were significantly lower than overall survival rates of pediatric and adult recipients, respectively.

The survival rates were compared among three most common liver diseases of adult recipients (Fig. 3) and among those of pediatric recipients with different diseases (Fig. 4). Survival rates of adult recipients who had postnecrotic or cryptogenic cirrhosis, those with primary biliary cirrhosis, and those of primary sclerosing cholangitis were nearly identical (Fig. 3). In pediatric recipients, survival rates of children with biliary atresia, those of liver-based inborn metabolic errors, and those of postnecrotic or cryptogenic cirrhosis were also similar (Fig. 4).

The influence of hepatitis B antigen (HBsAg) upon survival after liver transplantation and the recurrence of hepatitis B in the graft liver were studied. Among 276 recipients who had postnecrotic or cryptogenic cirrhosis, 36 patients were postive for HBsAg and the remaining 240 were negative for HBsAg before transplantation. One- to 5-year survival rates of B virus carriers were 57%, 48%, 40%, 40%, and 40%, and those without the carrier state were 78%, 77%, 74%, 71%, and 71%. Thus, survival rates of the patients with HBsAg were significantly worse than those of the patients without HBsAg (Fig. 5).

Recurrence of hepatitis B was examined among 24 patients who had had HBsAg-positive postnecrotic cirrhosis before transplantation and who survived more than 3 months after transplantation. Five of the 24 patients died between 4 and 15 months, and four of the five died because of recurrent hepatitis B. Eleven of the 24 patients are alive between 6 months and 6 years with or after recovery from hepatitis B recurrence. Eight of the 24 patients are alive without recurrence between 4 and 12 months. Only one patient has become HBsAg negative after transplantation during a short observation period of 7 months. This patient had perioperative alpha interferon therapy.

Fifty-three of the 1,000 patients received liver transplantation in the presence of primary hepatic malignancy other than bile duct carcinoma. Transplantation for 36 of these 53 patients was primarily to treat advanced hepatic malignancy that could not be resected by conventional techniques of subtotal hepatectomy. Eighteen of the 36 patients had hepatoma, eight had fibrolamellar hepatoma, eight had epitheloid hemangioendothelioma, one had cholangiosarcoma, and another had angiosarcoma.

For 17 of the 53 patients, liver transplantation was primarily to treat liver failure, and the malignant neoplasm in the diseased liver could have been resected by subtotal hepatectomy, if the liver was not terminally diseased. Fifteen of these 17 patients had hepatoma, one had fibrolamellar hepatoma and another had mixed cholangiohepatoma.

One- to 5-year survival rates of the 36 patients who received liver graft for the principal indication of malignancy were 65%, 50%, 29%, 29%, and 29%, respectively (Fig. 6). The survival rates of 18 patients with hepatoma were lower than those of eight patients with fibrolamellar hepatoma and those of eight patients with epitheloid hemangioendothelioma (Fig. 7).

Recurrence of primary hepatic malignancy after liver transplantation was examined among 27 of the 36 patients, who survived more than 6

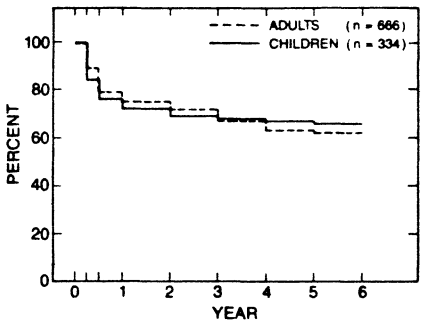

Fig. 2.— Nearly identical survival rates between 666 adult recipients and 334 pediatric recipients since 1980.

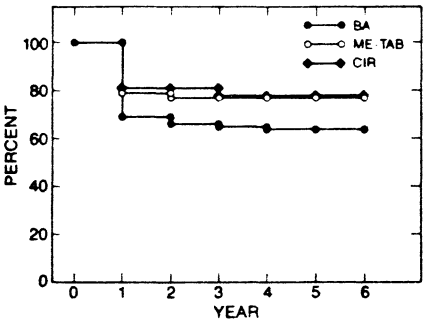

Fig. 4.— Survival rates of pediatric recipients with liver-based inborn metabolic errors were nearly identical to those with postnecrotic or cryptogenic cirrhosis. Survival rates of children with biliary atresia were slightly lower than the other two diseases, but the difference was not statistically different.

months. Tumor recurrence was observed in 13 of the 27 6-month survivors. Seven of 11 patients with hepatoma had tumor recurrence, and three of which occurred within 1 year. Three of seven patients with fibromellar hepatoma had recurrence. All of the recurrences were diagnosed more than a year after transplantation. Similarly, the two (of eight) patients with epitheloid hemangioendothelioma had recurrences more than a year after transplantation. One patient with cholangiocarcinoma died from metastases 31 months after transplantation, 8 months after tumor recurrence.

Of the 17 patients who received liver grafts primarily for liver failure and whose livers harbored hepatic malignancy, only one died from recurrence of hepatoma. The remaining 16 patients are alive, free of tumor between 3 months to 6 years. The chronic survival of 17 of 17 patients, with only one late death is noteworthy.

There were ten recipients who had carcinoma of the bile duct. In eight patients bile duct carcinoma was associated with primary sclerosing cholangitis, and in the remaining two patients the lesions were the so-called Klatskin tumors. One patient with Klatskin tumor died within a month from transplant complications, and another died from recurrence 8 months after transplantation. Of the eight patients who had bile duct carcinoma associated with sclerosing cholangitis, one died from transplant complications within 1 month, four patients died from tumor recurrence in 4, 12, 14, and 16 months after transplantation, and three patients were alive free of tumor in 12, 13, and 14 months. No patient with bile duct carcinoma has lived 2 years after transplantation in this series.

There were seven patients who received liver transplantation for secondary hepatic malignancy. Three of the seven patients died. The first patient with carcinoid tumor of the small intestine died from transplant complications within three months, the second patient with carcinoid tumor of the small intestine and adenocarcinoma of the bile duct died in 6 months from recurrence of the bile duct cancer, and the third patient with adenocarcinoma of unknown primary site died from recurrence 21 months after transplantation. The remaining four of the seven patients are alive and free of tumor. One patient with glucagonoma, which was initially diagnosed as a hepatoma with lymphnode metastasis, is alive after 21 months, and another patient with glucagonoma is alive 10 months after transplantation. One patient with gastrinoma is alive free of tumor in 2 months. One patient with leiomyosarcoma of the small intestine is alive, but with recurrent tumor after 15 months.

Discussion

Our first comprehensive report on liver transplantation using cyclosporine-steroid therapy in 1982 involved only 67 patients (41 adults and 26 children), but it had already predicted a vast improvement of survival after liver transplantation. The article had a strong influence upon the practice of hepatology.[1] Five years later, this report, involving 1,000 patients, confirms the earlier assessment. The survival rates of 74% at 1 year and 64% at 5 years are convincing enough to consider liver transplantation as an ultimate therapy for various end-stage liver diseases. Unless agents that are more effective and safer than cyclosporine are used, further significant improvement of overall survival may not be expected, because liver transplantation will be applied for patients of even higher risk than now. However, substantial improvements can be achieved in some areas.

The survival rates after liver transplantation among children under 1 year of age are lower than those of older children. One of the main reasons for this is technical difficulty due to the small structures. The incidence of hepatic arterial thrombosis is higher in this age group than older groups. Further technical refinements, intraoperative use of electromagnetic flowmeters, restricted use of perioperative coagulation factors and a proper anticoagulation therapy will reduce the incidence of technical failures. The lack of small sized pediatric organ donor is another reason of lower survival rates in this age group. Public education to raise awareness of

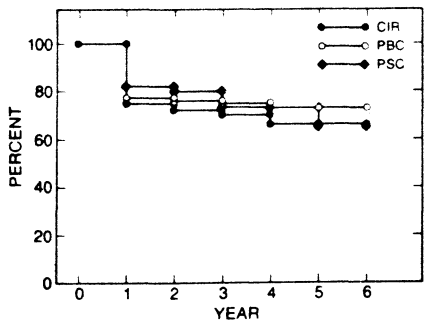

Fig. 3.— Survival comparison among adult recipients with three most common diseases (postnecrotic cirrhosis, primary biliary cirrhosis, and primary sclerosing cholangitis) did not show any statistical difference.

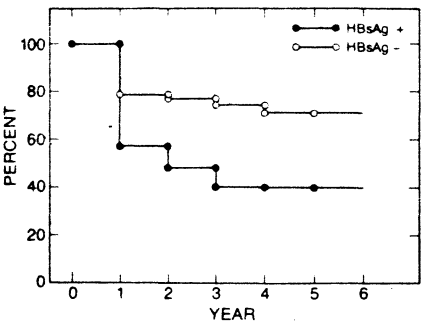

Fig. 5.— Survival rates of patients with HBsAg-positive, postnecrotic cirrhosis were significantly lower than those with HBsAg-negative postnecrotic cirrhosis.

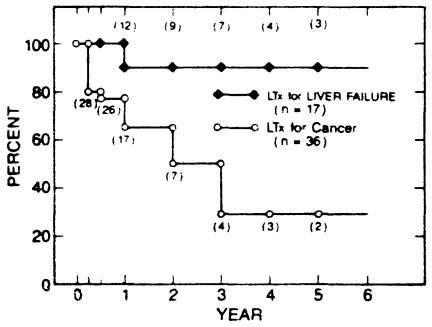

Fig. 6.— Survival rates of patients who received transplantation for primary liver malignancy were poor. However, survival rates of patients who had transplantation primarily for liver failure and whose advanced cirrhosis contained small malignancies were excellent.

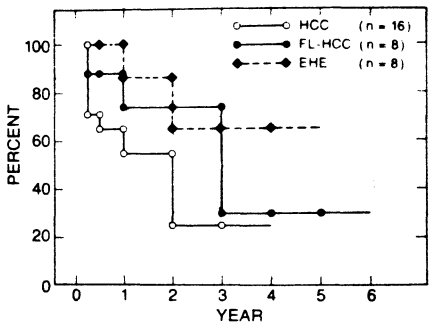

Fig. 7.— Survival rates of patients with hepatoma (HCC) were significantly lower than those with fibrolamellar hepatoma (FL-HCC) and those with epitheloid hemangioendothelioma (EHE).

urgent need of organ donation must be continued. Reduced size liver grafting, such as left lateral segment of a larger liver into a small pediatric recipient, can be carried out without significant additional risk as reported in this forum. This approach should decrease the waiting period for a proper donor organ, and thus the operation can be performed before an infant becomes too sick to have successful transplantation.

The survival rates of patients older than 60 years old are also lower than those of younger adults. Further experience in selecting elderly candidates will establish more accurate criteria of adequate cardiovascular, pulmonary, and neurologic functions for liver transplantation.

There were no statistical differences in survival rates among the most common diseases in adult recipients (postnecrotis cirrhosis, primary biliary cirrhosis, and primary sclerosing cholangitis) and among those in pediatric recipients (biliary atresia, liver-based inborn metabolic errors, and postnecrotic cirrhosis). However, slightly lower survival rates among children with biliary atresia are directly or indirectly caused by an unsuccessful portoenterostomy and unwise attempts of its revision before transplantation. Although many children with extrahepatic biliary atresia have been saved by portoenterostomy when it was done by proper technique while the liver was still relatvely normal, there are many other unfortunate children whose condition deteriorated rather rapidly because of unsuccessful attempts of the operation. It is not unusual that a child had had several abdominal operations, such as attempts of portoenterostomy, revisions of enterostomy, drainage of abdominal abscesses, and operations for bowel obstruction before transplantation. It is also not unusual that children with intrahepatic atresia or other nonextrahepatic type of biliary atresia, such as Alagille's syndrome, had a portoenterostomy and that children with well established cirrhosis and portal hypertension have received the operation. The portoenterostomy should only be performed by experienced surgeons at the institutes where jaundice-free success rate has been more than 70%. As liver transplantation has become a practical option for children with biliary atresia, indications of portoenterostomy and its rare revision must be strictly followed.

The survival rates of patients with hepatitis B surface antigen positive cirrhosis were significantly lower than those of patients with HBsAg-negative postnecrotic cirrhosis. Recurrence of viral hepatitis B was a general rule despite our efforts at perioperative use of anti-hepatitis B immunoglobulin therapy and ongoing alpha interferon therapy. The recurrence of hepatitis B was the direct or indirect cause of death in four of five late deaths among the 36 patients. It is worthwhile to note that 12 of the 36 patients died within 3 months from various complications related to transplantation, but not from recurrence of hepatitis B. Actually, this high early mortality among chronic hepatitis B virus carriers influenced the survival rates more significantly than the mortality related to recurrent hepatitis B. Immunologic impairment of chronic HBsAg carriers could play a significant role in early mortality. Thus, further investigations are needed not only for antiviral therapy, but also for the means to decrease early mortality that was probably not directly related to hepatitis B virus.

Recurrence of hepatic malignancy after transplantation resulted in lower survival rates of patients who received liver grafts for cancer than those of patients without hepatic malignancy. However, the mere presence of a small, incidental hepatic malignancy in the advanced cirrhosis did not influence the survival rates. When liver transplantation was performed to treat a large hepatocellular carcinoma that could not be resected by a conventional technique of subtotal hepatectomy, the tumor all too frequently recurred within 1 year. For the last few years, adjuvant chemotherapy with adriamycin has been applied for these patients as soon as the condition of the patients and the graft became stable, usually 1 or 2 months after transplantation. During this small and short exercise, there has been at least no significant adverse effects of adjuvant chemotherapy. Safer and more effective adjuvant chemotherapy and/or immunotherapy are needed to prevent tumor recurrence. The prognosis of fibrolamellar hepatoma and epitheloid hemangioendothelioma are better than that of hepatoma, as indicated by better survival rates, and later and lower incidence of recurrence.

In this series of 1,000 patients, there has been no 2-year survivor who had bile duct cancer. The recurrence of the tumor within a year has been frequent. The facts that bile duct cancer was found in eight of 81 patients with primary sclerosing cholangitis (10% incidence) and that the survival of these eight patients was extremely poor strongly indicate the need of earlier transplantation for the patients with long standing sclerosing cholangitis.

In general, metastatic liver tumors constitute a poor indication for liver transplantation. However, over the years, seven such patients had liver replacement accidentally or knowingly because of an unusually indolent clinical course. The survival rates of these highly selected patients were better than generally expected, particularly if the metastases are from pancreatic islet tumors and from carcinoid tumors.

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Clio, Greek Muse of History

Each volume in the series, *Clio Chirurgica,* is composed of papers that were important in the development of ideas in a sub-specialty area of surgery. This volume, *Liver Transplantation,* is the fifteenth in the series. It is unique because the field is new and because the primary author, Thomas E. Starzl, has been involved in the development of liver transplantation from its inception.

The events all are recent—they took place during the past 32 years. The papers, preceded by the authors' inciteful comments, describe the problems posed by transplantation of such a complex organ as the liver.

First, there was the question of location. What were the advantages and disadvantages of an orthotopic versus a heterotopic graft? The result was surprising. Although the orthotopic graft proved to be best suited for clinical application, investigation of the causes for failure of the heterotopic (auxiliary) graft yielded important information regarding hepatic physiology. It opened an entire field of so-called "hepatotrophic physiology".

Secondly, there were the problems of graft preservation, donor selection, operative technique and immunosuppression. These issues, with the exception of operative technique with regard to veno-venous bypass, were addressed more or less hand-in-hand with renal transplantation and are described in detail in the companion volume in this series, *Renal Transplantation.* Yet, even after these problems were overcome, liver transplantation could not be applied widely in clinical practice. Immunosuppression was not adequate. But that changed with the introduction of cyclosporine. When it was administered in conjunction with steroids, the results of liver transplantation improved dramatically.

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